TAXONOMY OF GELIDIALES IN MALAYSIA BASED ON MOLECULAR AND MORPHOLOGICAL STUDIES

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ABSTRACT

The Gelidiales is an important group of red algae that serves as a source of commercial agar and agarose. This order comprises three families and ten genera, of which *Gelidium, Pterocladia, Pterocladiella* and *Gelidiella* are the main sources of high quality agar and agarose. The Gelidiales is a very complicated order and the traditional classification of the order has been changed considerably based on modern molecular studies. Peninsular Malaysia and East Malaysia with extended coastlines in the Indo-Malayan ecozone have considerable potential of seaweed resources with 396 species reported in the latest checklist of the Malaysian seaweeds. Only eight species of Gelidiales have been reported for Malaysia. High morphological plasticity in the order Gelidiales attributed to the diverse ecological conditions, has resulted in many misidentifications using traditional taxonomy based on morphology. Molecular studies using modern tools have provided much basic molecular data to overcome the taxonomical complication of the order Gelidiales. In this study we hope to have a better understanding of the taxonomy of Malaysian Gelidiales. A combination of morphological study, as the traditional method, and molecular analyses as the modern method, was conducted to answer the question: Can the combination of molecular and morphological studies increase the number of Malaysian Gelidiales species or not?" Extensive collection of Gelidiales from the coastlines of Malaysia including Pulau Pinang, Pulau Langkawi, Pulau Pangkor, Pulau Besar, Port Dickson, Morib, Johor Baharu, Sabah and Sarawak has provided many specimens of Gelidiales for this study. In molecular studies after extraction of total genomic DNA of specimens collected in this study, partial sequences of three molecular markers including, *rbc*L, *cox*I and LSU were amplified and resulted in 98 sequences of these three genes. Phylogenetic analyses of the resulting sequences based on three methods, Maximum-likelihood, Maximum-

parsimony and Bayesian inference, resulted in identification of thirteen morphospecies of Gelidiales from Malaysia. Four species were proposed as new species, including two new species in the genus *Pterocladiella* and two new species in the genus *Gelidium*. Two new records, *Pterocladiella beachiae* Freshwater, *Pterocladiella bartlettii* (W. R. Taylor) Santelices, one species of the genus *Aphanta* and three morphological forms of the genus *Parviphycus*, were the other new revelations of this study. Two previously reported species, *Pterocladiella caerulescens* (Kützing) Santelices & Hommersand and *Gelidiella acerosa* (Forsskål) Feldmann & G. Hamel were also verified based on both molecular and morphological studies. Two new records *Pterocladiella bartlettii* and *Pterocladiella beachiae*, which have already been reported from Caribbean Sea, southern and Central America, represent the first reports of these species from Southeast Asia and the Indo-Malayan ecozone. This study increased the number of Gelidiales in Malaysia from eight to 17 species and also provided detailed morphological data as well as molecular data on partial *rbc*L, *cox*I and LSU genes of the identified and studied species. These molecular markers can successfully differentiate the species of the Gelidiales at intraspecific, interspecific, intergeneric and family levels and additional studies based on these three markers may be able to resolve the problems of heterogenetic lineages such as *Capreolia, Ptilophora* and distant genera such as *Pterocladia, Aphanta* and *Parviphycus.*

ABSTRAK

Gelidiales merupakan sekumpulan rumpai laut merah yang penting sebagai sumber agar dan agarose komersial. Order ini merangkumi tiga famili dan sepuluh genus yang mana *Gelidium*, *Pterocladia*, *Pterocladiella* dan *Gelidiella* merupakan sumber utama agar dan agarose yang berkualiti tinggi. Gelidiales adalah sebuah order yang merumitkan dan order klasifikasi tradisional telah berubah besar berdasarkan kajian molekular yang moden. Semenanjung Malaysia dan Malaysia timur yang mempunyai pesisiran yang panjang di zon ekologi Indo-Malaya, mempunyai potensi sumber rumpai laut yang yang tinggi yang mana sebanyak 396 spesies telah dilaporkan dalam senarai semak rumpai laut Malaysia yang terkini . Hanya 8 spesies Gelidiales telah dilaporkan di Malaysia. Morfologi yang amat plastik dalam order Gelidiales berikutan kepelbagaian keadaan ekologi telah menyebabkan kesalahan pengenalpastian menggunakan taksonomi tradisional berdasarkan morfologi. Kajian molekular menggunakan peralatan moden telah memberikan banyak data asas molekular untuk menyelesaikan kerumitan taksonomi bagi order Gelidiales. Dalam kajian ini, kami berharap untuk mendapatkan pemahaman yang lebih baik mengenai taksonomi Gelidiales di Malaysia, kombinasi kajian molekular sebagai kaedah moden, telah dijalankan untuk menjawab soalan:"Bolehkah angka spesies Gelidiales di Malaysia dipertingkatkan melalui kombinasi kajian molekular dan morfologi?" Banyak spesimen Gelidiales telah diperolehi melalui pengumpulannya yang meluas dari pesisiran Malaysia termasuk Pulau Pinang, Pulau Langkawi, Pulau Pangkor, Pulau Besar, Port Dickson, Morib, Johor Baharu, Sabah dan Sarawak. Di dalam kajian molekular, selepas pengekstrakan genomic DNA keseluruhan bagi spesimen-spesimen yang dikumpulkan didalam kajian ini, jujukan DNA yang separa daripada

tiga penanda molekular termasuk *rbc*L, *cox*I dan LSU telah diampliflamikasi dan membawa kepada 98 jujukan daripada ketiga-tiga gen tersebut. Analis filogenetik hasil jujukan ini berdasarkan tiga kaedah iaitu Maximum- likelihood, Maximum-parsimony dan Bayesian inference menghasilkan tiga belas identifikasi morfospesis Gelidiales dari Malaysia. Empat spesies telah dicadangkan sebagai spesies baru termasuk dua spesies baru dalam genus *Pterocladiella* dan dua rekod baru dalam genus *Gelidium*. Dua rekod baru, *Pterocladiella beachiae* air tawar; *Pterocladiella bartlettii* (W. R. Taylor) Santelices; satu spesies dari genus *Aphanta* dan tiga bentuk morfologi dari genus *Parviphycus* adalah antara penemuan baru dalam kajian ini. Kehadiran dua spesies yang pernah dilaporkan sebelum ini iaitu *Pterocladiella caerulescens* (Kützing) Santelices & Hommersand dan *Gelidiella acerosa* (Forsskål) Feldmann & G. Hamel, juga telah disahkan berdasarkan kedua-dua kajian molekular dan morfologi. Dua rekod baru *Pterocladiella bartlettii* dan *Pterocladiella beachiae*, yang mana telah direkodkan dari lautan Caribean dan Amerika selatan dan Amerika tengah, mewakili rekod pertama bagi *P.bartelttii* sepsis ini di Asia tenggara dan zon ekologi Indo-Malaya. Kajian ini meningkatkan bilagan Gelidiales di Malaysia dari lapan ke 17 spesies dan memberikan data morfologi yang terperinci dan data molekular dari gen-gen separa *rbc*L, *cox*I dan LSU bagi spesies -spesies yang diidentifikasi dan dikaji. Penanda–penanda genetik tersebut berjaya memberikan perbezaan spesies-spesies Gelidiales di peringkat intraspesifik, interspesifik, intergenerik dan famili. Tambahan pula, kajian berdasarkan ketiga-tiga penanda genetik tersebut dijangka dapat menyelesaikan masalah keturunan yang heterogenetik seperti *Capreolia, Ptilophora* dan genus yang berhubungan jauh separti *Pterocladia*, *Aphanta* dan *Parviphycus.*

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µg	microgram
AFLP	Amplified fragment length polymorphism
AICc	Akaike Information Criterion
ANT	Antarctica
AUS	Australia
BI	Bayesian Inference
BIC	Bayesian information Criterion
BP	Bootstrap proportion
bp	Base pairs
*cox*I	cytochrome c oxidase
CSR	Costa Rica
DNA	Deoxyribonucleic acid
dNTP	deoxynucleotide triphosphate
FAO	Food and Agriculture organization
FRN	France
g	gram
GRC	Greece
GTR	General Time Reversible Method
HKY85	Hasegawa-Kishino-Yano, 85
HNK	Hong Kong
HW	Hawaii
IND	Indonesia

ITL	Italy
ITS	Internal transcribed spacer
JAP	Japan
KK	Kota Kinabalu
KN	KwaZulu-Natal
LSU	Large subunit of rRNA
LSU	Large subunit
MCMC	Markov Chain Monte Carlo Method
mg	milligram
ML	Maximum likelihood
ml	milliliter
MP	Maximum Parsimony
MRC	Morocco
MULTREES	Minimal length trees
MX	Mexico
MY	Malaysia
MYN	Myanmar
MZ	Mozambique
NC	New Caledonia
ng	Nanogram
NOR	Norway
NSW	New South Wales
NZL	New Zealand
OD	optical density

OMN	Oman
PA	Panama
PAUP	Phylogenetic Analysis Using Parsimony
PAUP	Polygenetic analysis using parsimony
PB	Pulau Besar
PCR	Polymerase chain reaction
PD	Port Dickson
PHL	Philippines
PLN	Pulau Langkawi
PP	Pulau Pinang
PRT	Puerto Rico
RADP	Random amplified polymorphic DNA
*rbc*L	large subunit of Rubisco
*rbc*S	small subunit of Rubisco
rDNA	Ribosomal deoxyribonucleic acid
RFLP	Restriction fragment length polymorphism
RNA	ribonucleic acid
rRNA	Ribosomal ribonucleic acid
Rubisco	ribulose 1, 5 bisphosphate carboxylase/oxygenase
SAF	South Africa
SING	Singapore
SKO	South Korea
SPN	Spain
SSU	small subunit

TAN	Tanzania
TBR	Tree Bisection Reconnection
THI	Thiland
TK	Teluk Kemang
TRN	Terengganu
TX	Texas
UK	United Kingdom
USA	united States of America
UV	ultra-violet
VIT	Vietnam
VNZ	Venezuela

1.0 INTRODUCTION

1.1 An Introduction to Red Algae (Rhodophyta)

Seaweeds are classified into three broad groups based on their pigmentation: (1) brown seaweed (Phaeophyceae); (2) red seaweed (Rhodophyta) and (3) green seaweed (Chlorophyta). The red algae (Rhodophyta) are an ancient group of eukaryotic algae (Lee, 2008). There are over 10,000 species of red algae (Woelkerling, 1990; *Seo et al.*, 2010). Only two percent of red algae are found in fresh water and the rest are in the ocean. Members of red algae are mainly found in the tropical and temperate regions and values of 3.5-4.3 were reported as the ratio of red algae species to brown species (R /P) for tropical regions (Lünning, 1990).

Rhodophyta are a group among the photosynthetic eukaryotic, with chloroplasts lacking external endoplasmic reticulum and unstalked thylakoids (Woelkerling, 1990). They are characterized by absence of flagella and centriole structure in their cells (Pueschel, 1990). Phycoerythrin, phycocyanin, and allophycocyanins are the accessory pigments in their chloroplasts and chlorophyll *a* is their main chlorophyll content (Maggs *et al*., 2007; Woelkerling, 1990). Stalked phycobilisomes (van den Hoek *et al.*, 1995) are special organelles in red algae which are composed of various types of phycobiliproteins and polypeptides that are the light-harvesting system in the Rhodophyta. Storage polysaccharide in red algae is floridean starch in the cytoplasm, whereas in green algae and other plants polysaccharide storage products are accumulated in starch grains in the chloroplast (Sze, 1998; Maggs *et al.,* 2007). In the current classification of algae and based on modern molecular studies Rhodophyta are classified in the kingdom Plantae and Rhodophyceae is recognized as an individual class in the phylum Rhodophyta that is divided in two subclasses, Bangiophycidae, with 3 to 4 orders, and Florideophycidae with 21 orders (Saunders and Hommersand, 2004).

Bangiophycidae was classified based on spore formation mode (Rintoul *et al.*, 1999; Hommersand and Fredericq, 1990; Magne, 1989) and and organelle ultrastructure (Müller *et al*., 2001, 2002; Broadwater and Scott, 1994; Cavalier-Smith, 1998) and its orders are distantly related, but Florideophycidae is monophyletic and traditionally has been classified based on life history and sexual reproduction (Kylin, 1956; Scott and Broadwater 1989) and recent studies based on phylogenetic analyses has more clarified taxonomy of Rhodophyta (Saunders and Kraft, 1994; Saunders and Kraft, 1996; Saunders & Bailey, 1997; Saunders and Hommersand, 2004; Harper and Saunders, 2004; Saunders et al., 2004).

1.2 Importance of Red algae

Red algae among other groups of seaweeds are the best sources for food production and have wide range of uses in food, paper and textile industries, medicine, and even in building industries. Rhodophyta are main sources of phycocolloids and contain important chemicals such as agar, agarose, carrageenan, porphyran, furcelaran and other phycocolloids. These chemicals are commercially used worldwide, and their demand is increasing daily. The food industry is the first market for the seaweed phyocolloids (or hydrocolloids) where they are used as thickening and stabilizing agents; although agar and its derivative products, agarose and bacteriological agar, have long enjoyed attractive markets as microbiological and electrophoresis media, respectively.

The world production of seaweeds was reported to be about 1.8×10^7 tonnes in 2010 (FAO, 2012). The seaweeds are used in the production of food, feed, chemicals, cosmetics and pharmaceutical products. These plants are mainly utilized for the

production of food and the extraction of hydrocolloids. Seaweeds are mainly produced in Asian countries such as China, Philippines, North and South Korea, Japan and Indonesia. The USA, Canada and European countries such as France, Germany and the Netherlands are attempting to establish large-scale seaweed cultivation (Carlsson *et al*. 2007).

Gelidium and *Gracilaria* are two important genera as raw materials for agar resources. The best quality of agar is extracted from *Gelidium* species. All *Gelidium* used for commercial agar extraction comes from natural resources, principally from France, Indonesia, the Republic of Korea, Mexico, Morocco, Portugal and Spain (McHugh, 2003).

About 9600 tonnes of agarophyte red algae with sale value of US$ 173 million was produced in 2009 and its annual production growth rate has been estimated as 2.5% (Bixler and Porse, 2011). The main sources for production of agar in recent years are *Gracilaria* and *Gelidium.* The extracted agar from *Gracilaria* is preferred for food grade agar and its large scale cultivation has been successfully carried out in Chile and Indonesia (Bixler and Porse, 2011). Bacteriological and pharmaceutical grades of agar and agarose are derived from the species of Gelidiales especially *Gelidium*, *Pterocladia* and *Pterocladiella* (Bixler and Porse, 2011).

Red algae also have had a long history as food resources in Asian countries. A famous red seaweed used as food is the genus *Porphyra*. Nori and laver are common names for species of this genus. The dried forms of the plant in purplish black sheets are sold in markets of different Asian countries especially in China and Japan and are used for wrapping of cooked rice and fish. Japan and Korea have been growing *Porphyra*

(nori) since the seventeenth century. China, Japan and Korea are main *Porphyra* producers in the world and around 1000,000 tonnes fresh weight were produced in these three countries in 1999 (Bixler and Porse, 2011). The price of this seaweed is the highest among cultivated seaweeds, about US$ 1200 per wet tonne, compared with the brown seaweeds used as food that are valued at US$ 610/wet tonne for *Laminaria* and US$ 530/wet tonne for *Undaria* (McHugh, 2003). The recent reports on seaweed production showed about 85-88 % of world production of seaweeds belong to Asian countries, with Indonesia and Philippines being the two top producers of red algae (Neish, 2009; FAO, 2010).

Gelidiales are not recognized as important sources of agar and agarose and also as the only source of fine fiber (Seo *et al.*, 2010) in Malaysian seaweed resources. Only eight species of Gelidiales have been reported from Malaysian coastlines (Silva e*t al.*, 1996; Phang *et al*., 2007) without any detailed report about their morphology or molecular aspects. In this study Gelidiales species of Malaysian coastlines were studied by using a combination of morphological studies and modern molecular techniques analyses using partial DNA sequences of three genes *rbc*L, *cox*I and LSU.

1.3 Objectives of the Research

i. To collect and record the distribution of Gelidiales species in Malaysia and produce a checklist of Malaysian Gelidiales.
ii. To provide morphological and molecular data of Malaysian Gelidiales species for systematic study
iii. To use a combination of morphological and molecular data for understanding interspecific and intraspecific relationships of Malaysian Gelidiales and their

phylogenetic relationships with members of Gelidiales from other biogeographical regions.

1.4 Hypothesis:

H_0: The combination of morphological and molecular studies will not change the diversity of Gelidiales in Malaysia.

$H_{1:}$ The combination of morphological and molecular studies will change the diversity of Gelidiales in Malaysia.

2.0 LITERATURE REVIEW

2.1 What are Red Algae?

The red algae are common seaweeds in tropical marine waters. Only a few taxa of red algae are found in freshwater. Some are small and simple filamentous, while most of them are multicellular and have a pseudoparenchymatous structures or complex filaments. Their cells have double layered cell walls with the outer layer containing pectic substances, from which agar, agarose, carrageenan or some other colloid substances are extractable. The internal layer of their cell wall is mostly composed of cellulose (Fritsch, 1945).

The unique and distinctive feature of Rhodophyta is possession of pit plugs. The cell division in red algae is not a complete division and in the cytokinesis a small pore is left in the middle of the partition between daughter cells and the pit changes to a pit plug by deposition of cytoplasmic substance in the wall of the gap connected to the cells (Maggs *et al.,* 2007, Pueschel and Cole, 1982). Detailed studies by Pueschel (1994) stated the pit-plugs with two cap layers were originated from the naked (without caps) pit-plugs.

Triphasic life-cycle is another distinctive feature in the main group of Rhodophyta. A triphasic life-cycle has an alternation of a sporophyte and gametophyte generation, with an additional phase carposporophyte stage parasitic on the female. The carposporophyte produces carpospores, which germinate to tetrasporophyte thalli that produce tetraspores. Germination of tetraspores gives rise to gametophytes. Biphasic lif-cycle is another type of generation in Rhodophyta which may be isomorphic or heteromorphic.

2.2 Classification of Red Algae

Traditional classification of red algae was based on the ontogeny of female reproductive characteristics (Maggs *et al*., 2007), but the scarcity of reproductive features in biphasic red algae (e.g. Bangiophyceae) resulted in less development in their classification (Dixon, 1973), whereas the classification based on female reproductive features are relatively well developed in the triphasic Florideophyceae (Maggs *et al*., 2007). Incorporation of life-cycle ultrastructure and new morphological characters resulted in the establishment of more orders in the red algae (Garbary *et al.,* 1980). The pit-plug is another character of red algae which has been used for ordinal classification of red algae (Pueschel and Cole, 1982).

Modern molecular techniques are currently used as an important tool for taxonomy of organisms including the seaweeds. Molecular studies of algae started since the mid-1980s. More common and frequently used molecular markers in phylogenetic studies of seaweeds are shown in Table 2.1 (Maggs *et al.*, 2007).

Use of the SSU marker in molecular studies confirmed the Pueschel Theory about the ancestral origin of the pit-plug in seaweeds and defined the relationships of the orders in the largest group of red algae, 'florideophyte' (Saunders and Bailey, 1997). Based on molecular studies, Rhodophyta are phylogenetically located in the kingdom Plantae. Saunders and Hommersand (2004) suggested the subkingdom Rhodoplanta for red algae, which are classified in three main clades.

Rhodophyta as the largest phylum in the subkingdom Rhodoplanta was subsequently subdivided into three subphylum including Rhodellophytina,

Metarodophytina and Eurhodophytina (Saunders and Hommersand, 2004; Schneider and Wynne, 2006; Wynne and Schneider, 2007, Wynne and Schneider, 2010).

Table 2.1: Phylogenetic markers and their frequency in several studies

Genum	**Marker**	**Type**	**Ref.**	**Frequency**
Nuclear	5S	Ribosomal DNA	Hori *et al.* (1985)	3
	18S	Ribosomal DNA	Bhattacharya *et al. (*1990)	62
	28S	Ribosomal DNA	Freshwater & Bailey (1998)	21
	ITS region	Two ribosomal spacer	Steane *et al.* (1991)	17
	Actin	Gene	Hoef-Emden et al. (2005)	1
Mitochondrial	*cox*I	Gene	Saunders (2005)	1
	*cox*2-3	Intergenic spacer	Zuccarello *et al.* (1999)	11
Plastid	16S	Ribosomal DNA	Olsen *et al.* (2005)	3
	*rbc*L	Gene	Freshwater et al. (1994)	77
	*rbc*S	Gene	Lee et al. (2001)	2
	Rubisco spacer	Intergenic spacer	Destombe & Douglas (1991)	22
	*psa*A	Gene	Yang & Boo (2004)	3
	*psa*B	Gene	Yoon *et al.* (2004)	1
	*psb*A	Gene	Seo *et al.* (2003)	4
	*psb*C	Gene	Yoon *et al.* (2002, 2006)	1
	*psb*D	Gene	Yoon *et al.* (2002. 2006)	1
	*Tuf*A	Gene	Yoon *et al.* (2004)	1
	URP marker	Gene and spacers	Provan *et al.* (2004)	1

(Maggs *et al.* 2007)

Rhodellophytina are composed of simple unicells or pseudofilaments, cells arranged in a row surrounded by gelatinous coverage. There is no sexual reproduction in this group of red algae (Bold and Wynne, 1978; Saunders and Hommersand, 2004; Yoon *et al,* 2006).

Metarhodophytina includes the filamentous or pseudoparenchymatous members which have a biphasic life-cycle (Saunders and Hommersand, 2004; Yoon *et al.*, 2006).

Eurhododophytina including the red algae with biphasic or triphasic life-cycle and presence of endoplasmic reticulum in mitochondria and Golgi system. This subphylum comprises two classes, Bangiophyceae and Florideophyceae (Saunders and Hommersand, 2004, Yoon *et al,* 2006).

Florideophyceae, as the largest group in subphylum Eurhodophytina, contains most of the taxa in the phylum Rhodophyta. These plants are complex, filamentous or pseudoparenchymatous and most of them are in warm marine waters. Most of the commercial phycocolloid producers of Rhodophyta are members of the Florideophyceae (Dellatre *et al.,* 2011).

The life-cycle in Florideophyceae is triphasic (*Polysiphonia*-type, Fig. 2.1) with generally isomorphic sporophyte and gametophyte phases, and the third carposporophyte which is a result of zygote development produced by oogamous fertilization of gametes. The zygote nucleus typically moves to the auxiliary cell and in the auxiliary cell, develops and forms a set of small filaments arising from the diploid spores and produces carpospores at their terminal end. The carpospores are released, germinate and grow to produce the pseudoparenchymatous sporophyte.

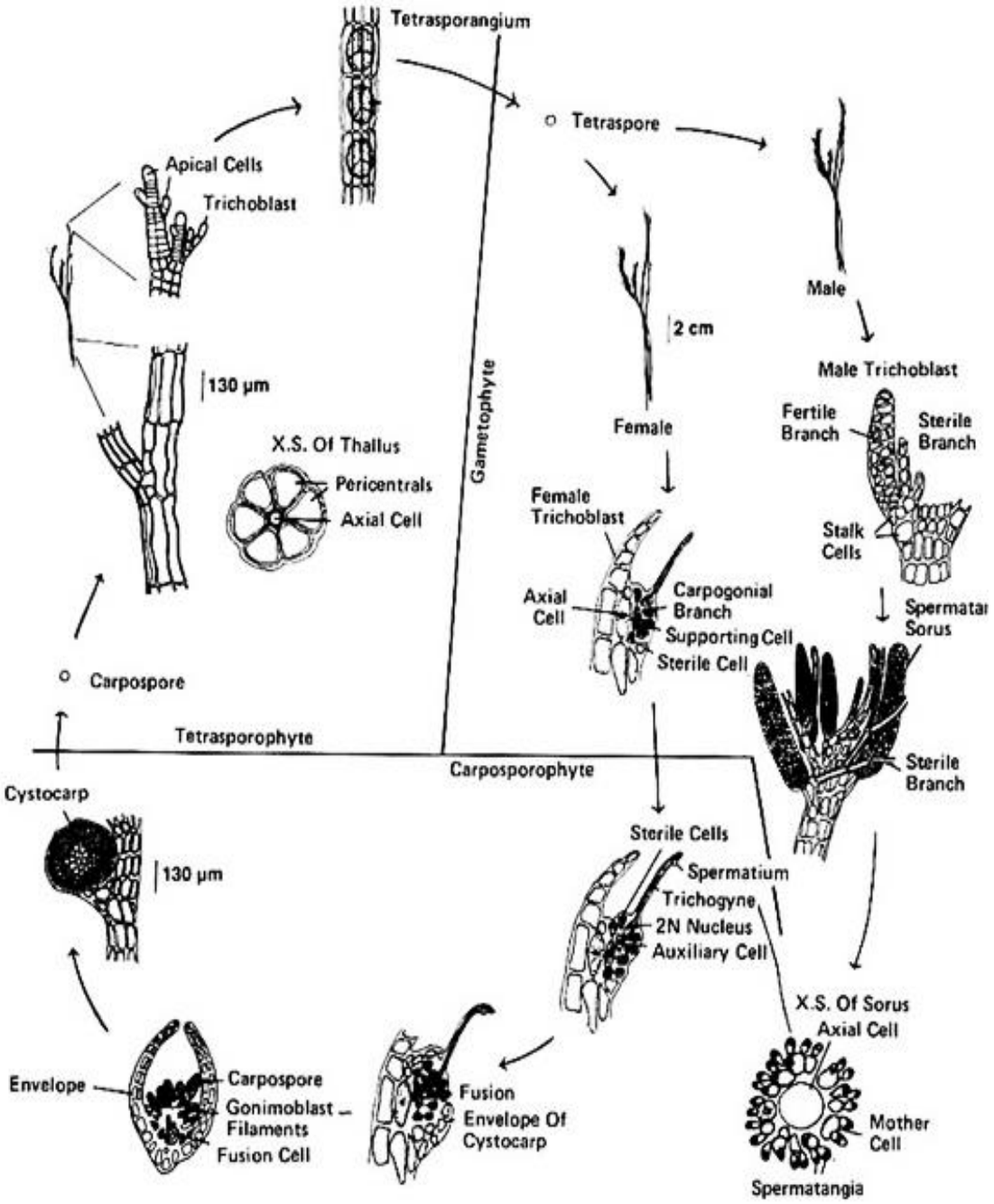

Figure 2.1: Triphasic or *Polysiphonia*-type life-cycle in red algae (Lee, 1980)

In sporophyte (tetrasporophyte) certain cells developed to sporangia (tetrasporangia) which produce tetraspores through meiotic divisions. The tetraspore germinates to form gametophytes. Male gametophytes produce spermatia in specialized spermatangia and females produce egg (carpogonia). A Trichogyne, an elongate hair like extension, is produced on each carpogonium and the nucleus of the spermatium when in contact with the trichogyne is transferred through the trichogyne and fuses with the egg nucleus. This is followed by the fertilized egg traveling to an auxiliary cell, where their development and growth lead to the new carposporophyte. The developed and matured carposporophyte produces a cup-like envelope, the cystocarp, around the carposporophyte (Saunders and Hommersand, 2004).

2.3 Economic Importance of Red Algae

Agar as a marine colloid is extracted from certain species of Rhodophyta. Agar is insoluble in cold water but soluble in boiling water to make a liquid which, when cooled, forms a firm, clear, resilient gel possessing suspending, stabilizing and thickening properties. The term agar has a Malayan origin and in that language refers to seaweeds such as *Gracilaria*, which produce a jelly used for making sweetmeats (McHugh, 1987).

Agars are usually composed of repeating agarobiose units alternating between 3-linked-β-D-galactopyranosyl and 4-linked-3,6-anhydro-α-L- galactopyranosyl units. About 90 percent of the agar product is for food applications, the remaining 10 percent being for bacteriological and other biotechnological uses. In the baked goods industry, the ability of agar gels to withstand high temperatures means agar can be used as a stabilizer and thickener in pie fillings, icings and meringues. Agar's use as a solid

substrate for the growth of bacteria and fungi is attributed to a laboratory assistant of Robert Koch (McHugh, 2003). No modern microbiological laboratory in the world can survive without agar, and no satisfactory substitute has been found even with today's technological advances (McHugh, 2003). *Gracilaria* species are the preferred seaweed for making food grade agar; because it has been successfully cultivated in Chile and Indonesia, and Gelidiales species are the preferred seaweeds for making bacteriological and Pharmaceutical grade agars and agarose. Global agar production in 2009 was estimated about 96,000 tonnes with the sale value of $173 million (Bixler and Porse, 2011).

The highest quality agar, and its derivative agarose, which are used for biotechnological and molecular investigation and gel electrophoresis, is extractable from special genera of seaweeds mainly from the species of *Pterocladia, Pterocladiella* and *Gelidium* belonging to the Gelidiales. Low sulfate content, high clarity and high gel strength in agarose compared to agar make it considerably more expensive (Laurienz, 2010). Food-grade agar which is mainly extracted from the species of Gracilariaceae is relatively cheap, around US$18 per kg, but the highest-quality agarose, comes from Gelidiales order is about US$5,000 per kg (http://www.seaweed.ie, Bixler and Porse 2011). Agar is used widely in genetic research. Pharmaceuticals use agar to regulate time-released medications (Saxena *et al.*, 2011).

Most plants of the Gelidiales species are collected from the wild resources in some countries, such as Spain, Morocco, Japan, México, and South Africa. Melo (1998) reviewed *Gelidium* exploitation, and showed that the most important *Gelidium*-based agarophyte resources are located in South Africa, Mexico and Chile.

2.3.1 Distribution and Commercial Importance of Gelidales

All the *Gelidium* used for commercial agar extraction comes from natural resources, principally from France, Indonesia, the Republic of Korea, Mexico, Morocco, Portugal and Spain (McHugh, 2003). The harvests of *Gelidium* are spread over a wide geographical area. Large quantities are harvested on the north coast of Spain, the middle to southern end of Portugal coastlines, and the west coast of Morocco (Bixler and Porse, 2011).

Quality and quantity of agar differ due to the seaweed source, seasonality, environmental conditions, reproductive status of the species, and karyological phase of plant, nitrogen content and also extraction method (Carter and Anderson, 1985, 1986; Santelices, 1988a, 1988b). A study on *Gelidium sesquipedale* showed the agar content varied around 40% of algal dry weight and reached a maximum of 44.5% in November. Agar gel strength was maximum in May and July (1000 g cm^{-2}), and melting (90 °C) and gelling (35 °C) temperatures varied slightly (Michael, 2008).

Environmental conditions can affect the agar quantity and quality of the species (Mouradi-Givernaud *et al.,* 1999). The agar content of *Gelidium robustum* ranged between 17.5 and 44.2% with two maximum values in summer and in winter while gel strength ranged between 515 and 665 g cm^{-2}, reaching a maximum during autumn (Freile-Pelegrín *et al.*, 1999). In some Gelidiales species, for example *Gelidium robustum* from California, agar content can be as high as 70% (Levering *et al.*, 1969).

Method of extraction also affects the quality and quantity of agar products. The agar yield from *G. serrulatum* and *G. floridanum* ranges from 12 to 34%, depending on the method of extraction. Agar extraction of both species directly with NaOH (0.25 N)

provided significantly higher gel strengths (>1100 g/cm^2) than either extraction in water or using an alkali pretreatment process of NaOH (1.0 N). Direct extraction of agar in NaOH (0.25 N) led to an improvement of gel strength by almost three times more than with extraction in water for *G. serrulatum* (Hernández *et al.,* 1995). Agar extractions from *P. capillacea* showed yields ranging from 12 to 32% (depending on extraction process) and gel strengths were highest when the samples were extracted after pretreatment in 1.0 N of NaOH. In all cases treatment with alkali led to a reduction in sulfate percentage of agar and an increase in 3, 6-anhydrogalactose (Lemus *et al.,* 1991). Studies on *Gelidiella acerosa* (Roleda *et al.,* 1997a, 1997b) showed higher agar content for vegetative plants while 3, 6-anhydrogalactose in tetasporophyte plant was significantly higher than vegetative plants. Bird and Hinson (1992) studied seasonal changes in agar content of four agarophytes including *Gelidium pusillum* that results showed gel strength significantly affected by season.

2.3.2 Gelidiales Cultivation

Gelidium cultivation in Korea and China started since 1989 (Melo, 1998; Fei and Huang, 1991). Growing of *Gelidium* has also been reported from Spain and Portugal and South Africa (Seoanc-Camba, 1997; Melo, 1998). Much effort and different methods have been carried out for growing of *Gelidium* spp. and *Pterocladiella* spp. (Seoane-Camba, 1997; Silva *et al.* 1998; Mercado *et al.*, 2001; Sousa-Pinto *et al.,* 1996). In most of these studies the results have shown that irradiance is an important growth factor.

Temperature is the main seasonal limiting factor for *Gelidium crinale* and has shown positive effect on weekly growth rate (Friedlander, 2008; Boulus *et al.,* 2007).

Water movement stimulates the growth of *G. robustum* (1% day^{-1} in 1.4 ms^{-1}). A treatment with greater dynamics could increase growth to 3.6% day^{-1} (Pacheco-Ruiz and Zertuche-Gonzalez, 1995).

Among chemical factors, ammonium is the main limiting factor and has positive effect on growth of Gelidiales (Boulus *et al*., 2007; Bird, 1976). *Pterocladiella capillacea* cultivation in tanks under outdoor conditions has shown the growth rate 28.3% and 12.5% per week when NH_4 and PO_4 were included once and twice a week for 24-h periods, respectively (Gal-Or and Israel, 2004). Phosphate concentration in laboratory condition can increase growth rate of *G. robustum* up to 21% d^{-1} in 150 µM (Sousa-pinto *et al*., 1996). Branching of vegetative fragments of *G. sclerophyllum* was stimulated by high phosphate concentration up to 100-150µM (Friedlander, 2008). High pH condition has shown negative effect on growth rate of *P. capillacea* and *G. sesquipedale* (Gal-Or and Israel, 2004; Mercado *et al.,* 1998). *Gelidium amansii* could grow in high density up to 1 $kg.m^{-2}$ (Friedlander 2008). In free floating tank culture density of *G. crinale* can be increased to 4 $kg.m^{-2}$ (Friedlander, 2008).

In free floating condition with fishpond effluent which is rich in $NH4^{+}$ a bioremediation experiment with *G. amansii* showed that it can be used as a biofilter (Liu *et al*., 2004). Generally studies have revealed that reproduction by attached and free-floating fragments is more effective than spore reproduction while a major problem in *Gelidium* cultivation is epiphyte infection (Friedlander, 2008).

2.4 The Gelidiales order

The order Gelidiales includes three families (Perrone *et al*., 2006), 10 genera (Santelices, 1990; Tronchin *et al*., 2007) and 140 (Santelices, 1976; Bouzon et *al*., 2007) to 208 species and varieties (www.algaebase.com, Guiry and Guiry, 2012). More than 50 species of the order are used as sources of good quality agar biopolymer (Santelices and Stewart, 1985; Santelices, 1990).

2.4.1. Morphology and Biology of Gelidiales

One of the important criteria which has been used for establishment of the order Gelidiales was life-cycle. Most algal life-cycles can be categorized in three general categories: (1) Gametic (diplontic), (2) zygotic (haplontic), and (3) biphasic or sporic/haplo-diplontic (Thornber, 2006; Lee, 1999).

Gametic (diplontic): in the gametic life-cycles, diploid organisms produce gametes through meiosis and the gametes are the sole haploid phase which rapidly fuse and create the diploid zygote. This type of life-cycle is the most common life-cycle in many animals (including humans).

Zygotic (haplontic): haploid organisms produce haploid gametes which fuse and produce diploid stage or zygote that rapidly generates haploid spores through meiosis. The haploid spore grows into new gametophytes.

Biphasic life cycle: in the biphasic life cycle haploid gametes are produced from mature, multicellular haploid gametophytes and released into the water column. Two gametes (usually male and female, or + and − in green algae) fuse and create a diploid zygote. After settlement zygote grows into a mature, multicellular diploid sporophyte.

Meiosis occurs when sporophyte produce haploid spores. These spores are released into the water column, settle, and then grow into new gametophytes (Lee, 1999).

A modified biphasic (or triphasic) life-cycle is a usual life-cycle in red algae which includes the third carposporophyte stage, a short-lived diploid, which is produced when fusion of male haploid gametes, which are released from the spermatangia, onto surface of the female gametophyte thallus (not into the water column) fuse to female gametes in the carpogonia and form diploid zygotes. The zygote lives attached to the female thallus and absorbs nutrient from the female thallus and forms a mass of diploid spores, carpospores that are released and settle on a substratum in water and grow to new free-living diploid, tetrasporophytes. In matured tetrasporophyte thallus, haploid tetraspores are produced by meiotic division. The tetraspores are released into the water, settle, and grow into male and female haploid gametophytes (Hawkes, 1990). Red algae lack flagellate gametes and triphasic life-cycle is an evolved mechanism for increasing reproduction output (Searles, 1980; Thornber, 2006).

Life-cycle of Gelidiales is triphasic with isomorphic gametophyte and tetrasporophyte. This triphasic life-cycle (as *Polysiphonia*-type life history, Fig. 2.1) for Gelidiales was proposed by Kylin (1923). Absence of functional auxiliary cell as a post-fertilization event was used for establishment of Gelidiales by Kylin (1923) and there was a general agreement on absence of auxiliary cell in cystocarp development in the Gelidiales (Santelices, 1990, 1999a). After fertilization the carpogonium gradually enlarges and fuses either with hypogenous cell (Fan, 1961; Kraft, 1976) or with neighboring cortical cells (Dixon, 1961; Hommersand and Fredericq, 1988).

Nutritive cell formations during cystocarp development, tetraspore germination pattern, architecture of apical cell, were other characteristics added to Gelidiales features by Papenfuss (1966) to better characterize this order. These characters were used by some authors to study some Gelidiales members at species level (Rodriguez and Santelices, 1993; Norris, 1992). Structure of pit-plugs, male gametes and spermatia formation method were additional characteristic of order Gelidiales (Pueschle and Cole, 1982; Santelices, 1990).

Absence or presence of auxiliary cell is an important criterion for classification at the subclass level but nutritive cells characteristic is a useful feature at the family or even genera level. This characteristic was used for Gelidiales establishment by Kylin (1923) and this character has been challenged in many studies (Kylin, 1928; Dixon, 1961; Fan, 1961; Hommersand and Fredericq, 1988; Papenfuss, 1951, 1955; Drew, 1954). There was a general agreement on absence of functional auxiliary cell in cystocarp development in Gelidiales (Santelices, 1990, 1999a). Santelices (1991) studied the characters of cystocarps as a criterion for distinguishing *Gelidium* from *Pterocladia*. He categorized different types of cystocarps in the two genera based on morphological features of cystocarp and introduced six types of cystocarps for these two genera and concluded that cystocarp architecture can be used for distinction in the two genera whereas Fan (1961) believed that structure and development process of cystocarp are similar in all genera of Gelidiales.

The apical cell as an initial cell for length growth in Gelidiales is another character in the order. An apical cell is a dome-shaped cell that basipetaly divides and produces a segment cell which after a subsequent longitudinal division, produces a central cell and two lateral pericentral cells; and the next division increases the number

of pericentral cells to four. The method of apical cell division was suggested as a distinctive character by Papenfuss (1966). This character has been used by Rodriguez and Santelices (1988) as a distinctive feature at the generic or even specific level. The distichous pattern of apical cell division was also used by Santelices (2004) to distinguish and establish the new genus *Parviphycus* in Gelidiellaceae, a finding that resulted in placement of eight *Gelidiella* species in the new genus (Santelices, 2004; Wynne, 2011), and was subsequently confirmed by molecular studies using *rbc*L gene sequences (Millar and Freshwater, 2005).

Tetraspore germination style is another important characteristic in Gelidiales. Kylian (1914) for the first time explained *Gelidium*-type germination as a pattern for spore germination in *Gelidium*. This pattern of germination was later described by different authors (Chemin, 1937; Ueda and Ketada, 1949; Ketada, 1955; Santelices, 1990) for many species of *Gelidium*, *Gelidiella*, *Pterocladia*, and *Acanthopeltis*. Tetraspore and carpospore both follow the same pattern of germination (Santelices, 1988a).

In this pattern of germination after the settlement of spore and its attachment on substratum, a germ tube is pushed out and the spore content evacuated into the germ tube subsequently and empty cell remains connected to the germ tube by a cross wall. By some divisions the initial sporeling is formed and after a number of divisions one or two rhizoids are produced and attach the sporeling to the substratum. The apical cell differentiates after a number of days and elongation continues by transverse division of the apical cell and its derivatives (Santelices, 1988a). Cytochemical changes during germination tube formation were studied by some authors (Boillot, 1963; Kaneko, 1966; Sreenivasa Rao, 1971a, 1971b; Echegaray and Soneoane-Camba 1996) who described

the details of the changes. Before the transfer of spore content to the germ tube, its protoplasm divides and forms eight nuclei by mitotic division. One of these nuclei (functional nucleus) migrates to the germ tube with cytoplasm of spore and the rest remain in the original spore and gradually degenerate during disintegration of the original empty spore. This ontogenic process of spore germination has been used by Papenfuss (1951) for more characterization of the order Gelidiales (Santelices, 1988a, 1990).

Spermatangia formation pattern was another criterion considered in Gelidiales taxonomy. Spermatangia formation is initiated by a transverse division in the parent cells, different from other red algae where the spermatangia are cut off by oblique division (Akatsuka, 1970, 1973, 1979; Gabrielson and Garbary, 1986; Santelices, 1990). During several studies on female reproductive structures, the processes of spermatagia formation were also studied in *Gelidium* and *Pterocladia* (Akatsuka, 1970, 1973, 1979; Fan, 1961; Santelices and Flores, 2004). All these studies, commonly assumed that *Gelidium* and *Pterocladia* are dioecious (Santelices and Flores, 2004).

Tetrasporangial stichidia are special and usually swollen, terminal branchlets that produce tetrasporangia in Gelidiales (Abbott and Hollenberg, 1976). In some species like *Parviphycus antipai* (=*Gelidiella antipi*) and *Parviphycus pannosus* (=*G. pannosa*) tetrasporangia arrangement is chevron shaped or in transverse rows whereas in *G. acerosa* tetrasporangia are arranged irregularly in swollen terminal stichidia. Some authors used *Gelidiella-type* and *Pannosa-type* to describe *Gelidiella* tetrasporangia (Perrone *et al*., 2006). In *Gelidiella fanii* stichidia was reported as club-like stichidia (Wiriyadamrikul *et al*., 2010; Lin and Freshwater, 2008). Even though Feldmann (1931) recognized that *Gelidiella* (as *Echinoaulon*) produced sporangia at swollen tips of

branchlets, he did not use this as a character to segregate *Gelidiella* and *Gelidium* nor did Fan (1961) when he segregated the family Gelidiellaceae.

Hyphae are unbranched, elongated fibers which have been called rhizines, rhizoids or intercellular fibers, and are formed near tips of axes at early stages of thallus development (Dixon, 1958; Fan, 1961; Feldmann and Hamel, 1934, 1936; Lee and Kim, 2003). Initial rhizines arise as small protuberances from the basal part of medullary cells and has dense cytoplasm and thin walls. They soon elongate and grow basipetally between the medullary or cortical cells and sometimes reach up to 200µm long. In older stages the cell wall is thickened, the lumen become narrow and the cell content decreases. They are assumed to provide structural support to the thallus (Feldmann and Hamel, 1936). In some species of *Gelidium* or *Pterocladia* and *Pterocladiella*, rhizines can be constant, but in others, rhizines disposition changes during the life history of the frond (Dixon, 1958). Lack of rhizine was the basis, Feldmann (1931) used to distinguish between *Gelidiella* and *Gelidium*. Maggs and Guiry (1987) suggested perhaps this lack is due to lack of routine search for hyphal cells in all parts of the plant and suggested that Gelidiellaceae be returned to the family Gelidiaceae. However recent molecular studies generally verified Gelidiellaceae as a monophyletic family (Perrone *et al*., 2006; Tronchin and Freshwater, 2007; Nelson *et al.,* 2006; Wiriyadamrikul *et al.,* 2010; Lin and Freshwater, 2008).

The morphology of attachment system and the characteristics of rhizoid ontogeny were verified as useful diagnostic and taxonomic features for vegetative thalli of Gelidiales members (Perrone *et al*., 2006). The genera and species belonging to Gelidiellaceae attach to substratum by independent unicellular rhizoids with exogenous origin that originate from external cortical cells of stolon surface. The genera and

species of Gelidiaceae attach to substratum by complex haptera (de Gregorio and Perrone, 1994; Perrone, 1994; Shimada *et al*., 1999, Perrone *et al*., 2006) which are true organs because they consist of cells with different origin, shape, cytology, development and function. The rhizoids have endogenous origin from internal cells of cortex and they have pit-connection with their mother cells. Species of *Pterocladia* and *Pterocladiella* have peg-like attachment system of rhizoids which are coalesced and embedded in a thick sheath (Perrone *et al.*, 2006; Santelices, 2007).

2.5 Taxonomy of Gelidiales

Many studies have been carried out on the taxonomy of Gelidiales. The genus *Gelidium* was introduced for the first time by Lamouroux (1813) based on the basic name *Fucus corneus* Hudson. Kützing erected family Gelidiaceae (Kützing, 1843) to accommodate the cartilaginous and pinnately branched algae which have fiber in their internal structure and produced cystocarps, small globose spermatia and cruciately divided tetrasporangia (Renfrew *et al.*, 1989). The family originally included some genera like *Acrocarpus* Kützing 1843, *Ctenodus* Kützing 1843, *Echinocaulon* Kützing 1843 and *Gelidium* Lamouroux 1813. Later other genera were added to the family including *Polycladia* Montagne 1847, *Thysanocladia* (Endlicher) Lindley 1846, *Delisea* Lamouroux 1819, *Chondrodon* Kützing 1847 and *Phacelocarpus* Endlicher et Diesing 1845. The family was reduced by J. Agardh (1851) and comprised four genera, *Gelidium* (contain subgroups *Acrocarpus, Ecinocaulon* (=*Gelidiella*) and *Gelidium*), *Pterocladia* J. Agardh 1851, *Suhria* J.Agardh ex Endlicher 1843 and *Ptilophora* Kützing 1847. Agardh used unilocular structure of cystocarp as the main feature to distinguish genus *Pterocladia* from the genus *Gelidium* which has bilocular cystocarps. Schmitz (1889) introduced 14 genera in five tribes for Gelidiaceae and added five more

genera to the family including *Harveyella* Schmitz et Reink, *Wrangelia* C. Agardh; *Atractophora* H.M.Crouan et P.L. Crouan, *Caulacanthus* Kützing and *Naccaria* Endlicher, causing Gelidiaceae to became a heterogenous family. *Porphyroglossum* Kützing, *Schottmullera* Grunow and *Spencerella* Darbishire were added to the Gelidiaceae. *Schottmullera* was later renamed to *Acanthopltis* by Okamura (Yatabe, 1892; Schmitz and Hauptfleisch, 1897; Renfrew et *al*., 1989). *Wrangelia*, *Naccaria* and *Atractophora* were removed into Wrangeliaceae (Oltmanns, 1904) and later *Harveyella* (Struch, 1926) and *Caulacanthus* (Feldmann and Hamel, 1934) were transferred to the order Gigartinales. De Toni retained *Wrangelia* in the Gelidiaceae and added *Haliacantha* J.Agardh, *Gulsonia* Harvey, *Yatabella* Okamura 1900 and *Gelidiocolax* Gardner to the Gelidiaceae (De Toni, 1924). The genus *Echinocaulon* was renamed to *Gelidiella* by Feldmann and Hamel (1934).

Kylin (1923) segregated the Gelidiaceae from the other families in the order Nemaliales and accommodated them into the new order, Gelidiales, because of nonfunctional auxiliary cell and diplobiontic life-cycle which was in contrast to absence of auxiliary cell and haplobiontic life-cycle in the order Nemaliales (Santelices, 1990). The Gelidiaceae was revised by Kylin (1956), who moved *Haliacantha*, *Gulsonia*, and *Spencerella* to the Ceramiales and added a new genus *Beckerella* Kylin to Gelidiaceae. Fan (1961) reviewed the order Gelidiales, kept eight genera in the family Gelidiaceae and erected a new family Gelidiellaceae to accommodate the genus *Gelidiella* based on the absence of rhizine and lack of sexual reproduction (Renfrew *et al.*, 1989).

Dixon (1961) returned Gelidiaceae to the order Nemaliales because he believed that dipolobiontic or *Polysiphonia*-type life-cycle in some of Gelidiales like *Gelidiella*

is questionable. Papenfuss (1966) by adding the characteristic features of nutritive chains and *Gelidium*-type spore germination redefined and warranted the ordinal position of Gelidiales. *Acropeltis* was merged with *Gelidium* by Santelices and Montalva (1983). Akatsuka (1986a, 1986b) erected two new genera, *Onikusa* and *Pterocladiastrum* with emphasis on vegetative charaters and morphology of cortex cells. Maggs and Guiry (1987) merged Gelidiaceae with Gelidiellaceae based on presence of rhizine in *Gelidiella calcicola;* however this suggestion was not followed by most phycologists. The *genus Capreolia* Guiry et Womersley was added to Gelidiaceae based on biphasic life-cycle (Guiry and Womersley, 1993). *Onikusa* and *Suhria* have been merged in *Gelidium* based on molecular study on *rbc*L gene data (Tronchin *et al.,* 2002). *Yatabella* was merged with *Acanthopeltis* by Shimada (1999) based on molecular studies on SSU, *rbc*L and ITS1 genes. *Pterocladiastrum* was suggested as synonym to *Pterocladia lucida* (Nelson *et al*., 2006, Guiry and Guiry 2012). *Beckerella* has returned to *Ptilophora* by Norris (1987) and his suggestion was supported by molecular studies on *rbc*L and LSU genes analyses (Tronchin *et al.*, 2003, 2004).

In spite of unilocular cystocarp in the type species, *Pterocladia lucida* (Brown ex Bornet) J. Agardh, evidence of unequal bilocular cystocarps in the species of *Pterocladia* (e.g. *Pterocladia capillacea*) led to establishment of the genus *Pterocladiella,* based on *Pterocladia capillacea* (S.G. Gmelin) Bornet (Santelices and Hommersand, 1997). Subsequently most species of genus *Pterocladia* and some species of *Gelidiella* and *Gelidium* were transferred to the new genus *Pterocladiella* (Santelices 1998, 1999a, 1999b, 2007; Santelices and Hommersand, 1997). In the family Gelidiellaceae, a new genus *Parviphycus* was proposed to place the species of *Gelidiella* with few regular rows of tetrasporangia, distichous pattern of apical cell division (Santelices, 2004). Based on attachment of prostrate system Perrone *et al.*

(2006) proposed a new family, Pterocladiaceae, to accommodate the species of Gelidiales with peg-like attachment haptera including *Pterocladia* and *Pterocladiella*, whereas the species of Gelidiaceae has brush-like attachment and Gelidiellaceae comprising the species with independent unicellular attachment haptera. In 2007, Tronchin and Freshwater introduced the new genus *Aphanta* from South Africa based on molecular data and prominent, robust stolon with both endogenous and exogenous origins of rhizoidal attachments, which was supported by analyses of molecular data of *rbc*L and LSU gene sequences but could not resolve the taxonomic position of the genus in three families of the order (Tronchin and Freshwater, 2007).

2.6 Problems of Traditional Taxonomy in Gelidiales

Traditionally identification and classification of the seaweeds have been based on morphological data of vegetative and reproductive structures. These data often are affected by environmental conditions and result in a variety of phenotypic forms of same species that may result in several names for the same species. This problem is evident in Gelidiales taxonomy and species in the order is renown as "being difficult to identify and even assign to genera" (Millar & Freshwater 2005), because most environmental factors such as light intensity, depth of water, water movement, wind and substratum can change phenotypic features of the species. Additionally small size of most Gelidialiales causes many problems in identification of this group of red algae, especially in vegetative phase of their life cycle. This problem can cause misidentification of these algae because in some geographic area, populations of Gelidiales are entirely vegetative or tetrasporic (Perrone *et al*. 2006) and tetrasporic sorus characteristics alone cannot allow proper assignment of these seaweeds either in

generic or specific levels (Maggs and Guiry, 1987; Guiry and womersley, 1992; Kraft and Abbott, 1998; Perrone *et al.,* 2006).

Reproductive structures especially cystocarp characters were the only valid tested criteria that can distinguish members of Gelidiales at the family and generic levels (Santelices, 1976). These characteristics have restricted use, because fertile female thalli are rare in most collections (Santelices, 1976). For comprehensive taxonomic decision about Gelidiales members, many authors have recommended that frequency of field studies are very important (Greville, 1830; Stewart, 1968) but this recommendation has been generally ignored and descriptions for the species of two common genera, *Gelidium* and *Pterocladia* were based on few herbarium specimens (Chapman, 1969; Santelices, 1997) that resulted in a large number of specific and subspecific taxa for these groups of algae (Santelices, 1976). Therefore due to the limitation of morphological features for proper identification, modern molecular studies based on genomic information that actually is closely linked to characters of species can help to resolve most of the problems in evolutionary classification of the species.

2.7 Molecular Techniques a Modern Tool for Seaweed Taxonomy

Molecular studies are modern scientific methods that can be used for assaying the biological events and phenomena at molecular scale (King and Stansfield, 1985). Development in molecular techniques has increased since the 1990s and has had important effect on phylogenetic studies of organisms including the algae and seaweeds (Brodie and Lewis, 2007; Hillis *et al.,* 1990). Commercial exploitation of important economic organisms need scientific classification for proper utilization and exploitation management. Due to the earlier mentioned limitations of morphological features in

identification, modern molecular approaches can play an important role in the taxonomy of seaweeds.

Different techniques for molecular analyses have been used for phylogenetic studies of seaweeds; random amplification of polymorphic DNA (RAPD), restriction fragment length polymorphism (RFLP), amplified fragment length polymorphism (AFLP) and nucleic acid sequencing are important methods in modern molecular studies.

2.7.1 **Polymerase Chain Reaction** (PCR)

The polymerase chain reaction (PCR) is a molecular method to amplify a single or a few copies of DNA pieces to generate thousands to millions copies of a particular DNA sequence. This technique was developed by Mullis (Mullis *et al.,* 1986). PCR is now a common and often indispensable technique used in medical and biological research laboratories for a variety of applications. These include DNA cloning, DNA-based phylogeny, functional analysis of genes, determination and recognization of hereditary diseases, identification of genetic fingerprints (used in forensic sciences and paternity testing), and the detection and diagnosis of infectious diseases (Saiki *et al.,* 1985, 1988).

Nucleic acid sequencing determines gene evolution, population genetic diversity, geographic variation, and interspecific relationships of organisms. Availability of specific primers for PCR amplification and sequencing for most of the organisms, resulted in the PCR amplification to be fast, cost effective and the most extensively used method. Nucleic acid sequencing is one of the effective methods to solve and

overcome the morphological and phenotypic variation problems in traditional taxonomy of Gelidiales.

2.7.2 DNA Sequencing

DNA sequencing is the most important molecular technique, this technique which has been used since 1970s, is a fast and efficient technique and the most used molecular approach for phylogenetic studies (Brown, 2002). Successful genetic studies initially depend on the success in the isolation and purification of high quality DNA. Therefore, improvement in molecular taxonomy is dependent on the development of nucleic acid isolation methods.

2.7.3 Random Amplified Polymorphic DNA (RAPD)

Random Amplification of Polymorphic DNA (RAPD) is another molecular technique where by a single short primer (8-12 nucleotides) is used for PCR reaction (Williams *et al.,* 1990). The RAPD technique allows us to differentiate between genetically distinct individuals, and analyse the genetic diversity of an individual by using random primers. RAPD is a fast and simple technique and is a commonly used for taxonomic studies of seaweeds at the genus and species level, viz. *Gracilaria* species (González *et al*., 1996), *Gelidium* species (Alberto *et al.,* 1999; Patwary *et al*., 1993, 1994, Patwary and Meer, 1998), *Porphyra* (Dutcher and Kapraun, 1994) and *Sargassum* species (Ho *et al*., 1995a, 1995b).

2.7.4 Restriction Fragment Length Polymorphism (RFLP)

RFLP has been used for DNA sequence divergence estimation, based on changes in bases of DNA fragments caused by deletions, substitutions or insertions of nucleotides across the entire genome. RFLP is a relatively low cost and simple method for evaluation of algae at the population, genus and species level in phylogenetic studies and has been used for species of *Gracilaria* (Goff and Coleman, 1988; Rice and Bird, 1990). For genetic diversity assay and genetic mapping of many species, the restriction fragment length polymorphism (RFLP) is a good technique (Botstein *et al*., 1980).

2.7.5 Amplified Fragment Length Polymorphism (AFLP)

AFLP is a method which combines the advantages of RAPD and RFLP methods into a powerful tool to produce information that appears useful for analyses from large biogeographic scales to smaller population-level studies. High purity isolation of DNA is essential for AFLP technique. The availability of many different restriction enzymes and corresponding primers allowed the generation of large number of bands which provide a high chance to finding a large number of polymorphic bands among them. This method is a combination of RAPD and RFLP method by selective PCR amplification to produce useful information for analysis of biogeographic features of population (Vos *et al.*, 1995).

2.7.6 Phylogenetic Analyses of Sequences

DNA sequence data provides a valuable tool for molecular systematics and comparative phylogenetic analysis. Construction of evolutionary and phylogenetic trees based on

nucleotide sequences help to solve the taxonomic problems of several organisms when morphological and phenotypic characters cannot be used satisfactory.

Parsimony analyses of *rbc*L nucleotide sequences were used to develop hypotheses of relationships among taxa in the taxonomically difficult orders such as Gelidiales (Freshwater and Rueness, 1994). Several statistical methods are used for sequence analysis. Maximum parsimony (MP), Maximum likelihood (ML), Neighbor-joining (NJ), Bayesian inference, etc., and many types of software are available for these analyses.

2.8 Molecular Studies of Gelidiales

Most molecular systematic studies on Rhodophyta have been based on sequences determined for two genes, small subunit rRNA (SSU) and the plastid-encoded large subunit of RuBisco (*rbc*L) (Bird *et al*., 1992; Saunders and Kraft, 1994, 1996; Freshwater, 1993; Freshwater *et al*., 1994a, 1994b, 1995; Fredericq *et al.*, 1996; Fredericq and Ramirz, 1996; Tronchin and Freshwater, 2000). SSU and *rbc*L have been proven proper for estimating phylogenetic relationships of different taxonomic levels (Freshwater, 1993; Freshwater *et al.*, 1994, 1995; Bailey and Freshwater 1997, Freshwater and Bailey 1998). These genes have shown good support in phylogenetic relationship studies of the order Gelidiales (Freshwater *et al*., 1995; Bailey and Freshwater, 1997; Freshwater *et al.*, 1994). The relationship of some genera and species in this lineage has not been clarified based on *rbc*L trees and did not support the present classification of the order and indicated that they are not monophyletic (Bailey and Freshwater, 1997; Freshwater and Rueness, 1994). SSU sequences analysis has resolved ten lineages of Gelidiales (Freshwater *et al.*, 1995). Bailey and Freshwater (1997) used

combined analysis of *rbc*L and SSU sequences that supported the results of phylogenetic relationships of the order by Freshwater *et al.* (1995).

Species of Gelidiales including seven recognized genera: *Capreolia, Gelidiella, Gelidium, Onikusa, Pterocladia, Ptilophora,* and *Suhria* were studied by Freshwater *et al.* (1995). In the molecular phylogenetic study using small subunit ribosomal DNA sequences, Neighbor-Joining (NJ) analysis and Maximum Parsimony (MP) analysis were used for taxonomic problems of some Gelidiales in Japan (Shimada and Masuda, 2000, 2002; Shimada *et al.*, 1999). Patwary *et al.* (1998) used 18S ribosomal RNA (rRNA), internal transcribed spacer 1 (ITS1), 5.8S rRNA, and ITS2 regions for understanding the molecular relationships of *Gelidium* and *Pterocladia/ Pterocladiella*. Phylogenetic analyses based on the 18S rRNA genes on *Gelidium* species, *Pterocladia lucida,* and *Pterocladiella capillacea*, confirmed the division between *Gelidium* and *Pterocladiea*/*Pterocladiella*, and verified the segregation of *Pterocladiella* from *Pterocladia* by Santelices and Hommersand (1997). Analyses of 18s rRNA, 5.8s rRNA genes, ITS1, and ITS2 regions also proved the separation of *Gelidium, Pterocladia* and *Pterocladiella*. This study based on also showed an extensive sequence divergence between these genera and depicted that the divergence between *Pterocladiella* and *Pterocladia* is more than the divergence between *Gelidium* and *Pterocladiella.*

Large subunit of rRNA encoded gene (LSU) and *rbc*L sequences data were analysed in 16 species of the order Gelidiales from Canary Island , Spain, (Rico *et al.*, 2002), and both analyses showed the *Capreolia* and *Ptilophora* are not monophyletic genera. Results of the combined analyses of LSU and *rbc*L (Rico *et al.*, 2002; Bailey and Freshwater, 1997) showed four distinct lineages in the order Gelidiales which were

strongly supported as monophyletic groups. This is correlated to the different ways of nutritive cell formation during cystocarp development.

Millar and Freshwater (2005) studied Australian Gelidiales by *rbc*L analysis. Results of this study also showed that *rbc*L sequences analysis can resolve all major clades of Gelidiales except *Capreolia* and *Ptilophora* species. Based on *rbc*L analysis in Gelidiales, four genera of this order were merged. *Onikusa* Akatsuka and *Suhria* J. Agardh ex Endlicher merged into *Gelidium* (Tronchin *et al.,* 2002) and *Beckerella* Kylin and one *Gelidium* sp. also merged into *Ptilophora* Kützing (Tronchin *et al.*, 2003, 2004).

Diversity and phylogenetic relationships of New Zealand representatives of the red algal order Gelidiales have been examined using *rbc*L sequence data (Nelson *et al*., 2006). Tronchin and Freshwater (2007) used LSU, SSU and *rbc*L for Gelidiales species of South Africa and introduced the new genus *Aphanta* in this order. Genus *Pterocladiella* in Korea also studied based on *rbc*L sequences (Boo *et al.,* 2010). Molecular studies on monospecific genus *Porphyroglossum* Kützing in Indonesia based on *rbc*L and *cox*1 genes merged the genus into the genus *Gelidium* (Kim *et al.,* 2011). Combined morphological studies and molecular analyses on *rbc*L and *cox*1 genes led to identification of five new species of genus *Gelidium* (Kim *et al.,* 2011, 2012). Molecular studies on population of *Gelidium* verified the restricted distribution of *Gelidium pusillum* in Europe and USA (Kim and Boo, 2012). Combined molecular studies using *ps*A, *rbc*L and *cox*I genes also verified that *G. pusillum* does not exist in Southeast Asia (Kim *et al.,* 2011).

2.9 Description of the Gelidiales Order

The Gelidiales is composed of cartilaginous plants attached to the substratum by fibrous to discoid holdfasts, thallus may be attached by brush-like or peg-like rhizoidal holdfasts; transverse and subsequent longitudinal division of apical cell result in the growth and development of plant; terete to compressed or flattened erect axis; subdichotomously to irregularly or pinnately branched, lateral filaments paired from each axial cell in longitudinal section, distichously or decussately arranged, these branching to form cortex where cells interconnect with cells from adjoining filaments through secondary pit connections; cortical cells are relatively small and closely packed, the inner cortex or medulla is marked by thickened internal rhizoids (hyphae or rhizine). Reproductive structure located in determinate fertile branches. Tetrasporangia embedded, usually cruciately divided; if bisporangial, with two nuclei per spore. Sexual plants are dioecious, rarely monoecious. Female gametophytes with notch at fertile branch tips and longitudinal furrow along which developing female reproductive structures lie. Carposporophytes surrounded by thick pericarp with one or more ostioles; carposporangia large, arranged in chains or single. Male gametophytes bearing spermatangia in colorless patches (Abbott, 1999). The Gelidiales include three families, ten genera and 208 accepted species (Guiry and Guiry, 2012), (Table 2.2).

Table 2.2: Families, genera and number of species in the order Gelidiales.

Family (no. species)	**Genera (no. species)**			
Gelidiaceae (165)	*Acanthopeltis*(3)	*Acrocarpus**	*Acropeltis**	*Beckerella* (1)
	Gelidium (143)	*Onikusa**	*Capreolia* (1)	*Porphyroglossum**
	*Ptilophora (*17)	*Suhria**	*Yatabella**	
Pterocladiaceae (20)	*Aphanta* (1)	*Pterocladia* (5)	*Pterocladiastrum**	*Pterocladiella* (14)
Gelidiellaceae (23)	*Echinocaulon**	*Gelidiella* (17)	*Parviphycus*(6)	

(*genera merged with other taxa of the order or other orders) (Source of data Guiry and Guiry, 2012).

The current accepted genera in the families, based on the latest changes, are listed and explained below.

2.9.1 Family Gelidiaceae Kützing, 1843

Plants uniaxial, erect axes arising from fibrous holdfast, the holdfast solitary or clumped or stoloniferous; creeping axes terete to compressed, frequently unbranched; erect axes usually compressed, with several orders of branching, densely branched when fertile. Rhizines common in medulla, inner cortex or both. Tetrasporangia cruciately divided, embedded in terminally indeterminate branchlets. Spermatangia in fertile leaflets. Cystocarps relatively conspicuous when mature, with mostly two equally divided locules in *Gelidium* (Abbott, 1999). The genera of this family which are accepted based on recent studies are as follows.

2.9.1.1 *Acanthopeltis* Okamura in Yatabe 1892

Synonym: *Yatabella* Okamura 1900.

Description: Thallus subcylindrical, sympodially branched with erect axes, attached to the substratum by several elongated haptera. Branching may be alternate or dichotomous. All the segments, except the basal portions, are covered with numerous spirally arranged disk-shaped, suborbicular, leaflike structures.The margins and surface of the leaflets may have many bristle-like projections. Tetraspores are formed in dilated processes near the margin of ramuli. Cystocarps are roundish-oval, produced in the marginal setae (Yatabe, 1892; Guiry and Guiry, 2012).

The genus *Schottmullera* which had been added to the family Gelidiaceae by Schmitz (1889) was later renamed to *Acanthopltis* Okamura in Yatabe (Yatabe 1892),

the monospecific genus *Yatabella* (*Yatabella hirsuta* Kylin) which was recognized by Kylin (1956), was merged into the genus *Acanthopeltis* (Shimada *et al.* 1999) as a new combination *Acanthopeltis hirsute* (Kylin) S. Shimada, Horigushi and Masuda, based on morphological and molecular studies. The species of the genus have been reported only from coastline of Japan from shallow subtidal rocky habitats. Currently three species of the genus has been recorded in Algaebase website (Guiry and Guiry, 2012) as follows:

1. *Acanthopeltis hirsuta* (Okamura) S.Shimada, T.Horiguchi & Masuda
2. *Acanthopeltis japonica* Okamura
3. *Acanthopeltis longiramulosa* Y. Lee & B. Kim

2.9.1.2 *Beckerella* Kylin 1956

Beckerella was separated from the genus *Ptilophor*a Kützing based on smooth surface of thalli which is aboundantly proliferate in the latter (Kylin, 1956). Observation of proliferation in young thalli of *Beckerella* even in the type species caused by ephiphytes and injuries of thalli, led to *Beckerella* being merged with *Ptilophora* by Norris (1987). Latter studies based on morphological features and molecular analysis of large-subunit ribosomal rRNA (LSU) and *rbc*L genes sequences confirmed *Beckerella* and *Ptilophora* to be in the same, strongly supported clade (Tronchin *et al.,* 2003). Morphological analysis also confirmed the all species named *Beckerella* including the type species have proliferations, a character that had been statated is absent in *Beckerella* (Kylin, 1956). List of the *Beckerella* species have been transferred to the genus *Ptilophora* is presented in Appendix1.

2.9.1.3 *Capreolia* Guiry et Womersley 1993.

Description: The thallus is brown to dark red-brown, cartilaginous; irregularly branched; forming a variously intricate mat on rock or shells. The stolons are terete with haptera consisting of congregated rhizoids, arising from below and often terminating in short branches commonly opposite erect branches. Branches terete to compressed, sometimes subdistichously branched near their apices, with laterals gradually contracting to a more or less conspicuous apical cell. Tetrasporangial stichidia are stipitate with a rounded apex and spherical to ovoid sporangia in regular, shallow, acropetal capreolate rows on both sides of the thallus. Only one species, *Capreolia implexa* Guiry & Womersley has been recognized in this genus.

In all molecular studies this genus was grouped as a sister clade with *Gelidium caulacantheum* J. Agardh (Freshwater et *al.,* 1994; Freshwater and Rueness, 1994; Freshwater and Bailey, 1998) and *Gelidium hommersandii* Millar and Freshwater (Millar and Freshwater, 2005; Nelson *et al.,* 2006). According to Nelson *et al.* (2006), the generic concept of *Capreolia*, based on life-history characteristics, needs to be modified to accommodate additional species possessing ‘‘*Gelidium*’’ life histories.

2.9.1.4 *Gelidium* J.V. Lamouroux 1813

Synonyms: *Acropeltis* Montagne 1839; *Acrocarpus* Kützing 1843; *Suhria* J. Agardh ex Endlicher 1843; *Onikusa* Akatsuka 1986.

Description: The thallus is cartilagineus, with one or several erect axes, terete or compressed, distichously, plumously or irregularly branched, red, deep purple, to blackish in color. Erect axes arise from cylindrical or compressed, branched or unbranched creeping axes with numerous short haptera forming massive disc-like

holdfasts. The erect fronds can be cylindrical at the base, subcylindrical above and frequently compressed at their apical ends. Rhizines or hyphae located in the medullary and/or cortical tissue. The tetraspores in the sori are located on the tips of lateral branches or main axes. Fertile branches can be simple or pinnately compound, with sterile margins. Tetrasporangia are cruciately divided, and arranged with or without order in the sori. Carpogonial filament is unicellular, fusing with adjacent cells after fertilization. The cystocarp protrudes equally on both surfaces of the branch, usually with one or several openings on each surface of the frond. Occasionally, two cystocarpic cavities coalesce laterally, forming enlarged cystocarps. *Gelidium corneum* (Hudson) J.V. Lamouroux is the type species of Gelidiaceae.

Santelices and Montalva (1983) offered evidence for the merging of *Acropeltis* within *Gelidium*. Tronchin *et al.* (2002) presented molecular and morphological data to support the congeneric status of *Suhria* and *Onikusa* with *Gelidium*. In the database of algae, www.algaebase.org, (Guiry and Guiry, 2012). 291 species and varieties were listed under *Gelidium* of which 143 species and 43 varieties are currently accepted (Appendix 2). The others were transferred to the genus *Pterocladiella* (Appendix 3), renamed and were suggested as synonym at the interspecific level in the genus *Gelidium* (Appendix 4), transferred to the genera of other orders (Appendix 5), transferred to *Gelidiella* (Appendix 6) and P*tilophora* (Appendix 7).

2.9.1.5 *Onikusa* Akatsuka 1986

Onikusa was established by Akatsuka (1986a) based on surface cell arrangement, tetrad aggregation of cortical cells on apical and middle part of erect axes and presence of abundant proliferation, from studies on *Gelidium prestoides* from South

Africa and *Gelidium japonicum* from Japan and Taiwan. Based on molecular studies *Onikusa* and *Suhria* were later merged with *Gelidium* (Tronchin *et al.,* 2002).

2.9.1.6 *Porphyroglossum* Kützing 1847

Porphyroglossum was erected by Kützing (1847) with a monospecific species *Porphyroglossum zollingeri* Kützing from Indonesia. Based on molecular studies on *rbcL* and *cox*I genes, this species was resolved into the clade *Gelidium* and a new combination *Gelidium indonesianum* (Kützing) K.M.Kim, G.S.Gerung & S.M.Boo was proposed for the species (Kim *et al.,* 2011).

2.9.1.7 *Ptilophora* Kützing 1847

Description: The thallus erect, are arised from a rhizomatous-stoloniferous holdfast. Erect axes is repeatedly branched, with primary and secondary determinate and indeterminate branches; the branches blade-like and usually with a prominent midrib. Primary major branches arise from the surface and/or the margins of the main axis. Several layers of subsquare, small, pigmented anticlinally arranged cortical cells and spheroidal, larger, internal cortical cells. Rhizines are found interposed among medullary filaments and among the anticlinally arranged, cortical cells. Rhizines are scarce or absent from the thin blade regions. Reproductive structures are borne in sori on marginal proliferations or, rarely, from proliferations on the surface of blades or midribs. Cystocarps are spherical, bilocular, with one opening on each blade surface.The genus was known from temperate and warm waters with representative species in South Africa, Southern Australia, Philippines, Japan and the Mediterranean.

Norris (1987) presented evidence to merge *Beckerella* with *Ptilophora*. Tronchin et al. (2003) provided both morphological and molecular data to support the congeneric status of *Beckerella* and *Ptilophora.* Species of genus *Ptilophora* are shown in Appendix 8 and species of *Beckerella* have been synonym with *Ptilophra* are shown in Appendix 1.

2.9.1.8 *Suhria* J. Agardh ex Endlicher 1843

Based on the combined molecular and morphological studies by Tronchin *et al.*, (2002), the two genera *Suhria* and *Onikus* were merged with the genus *Gelidium*, and the type species of *Suhria*, *Suhria vittata* (Linnaeus) Endlicher is accepted as *Gelidium vittatum* (Linnaeus) Kützing (Guiry and Guiry, 2012).

2.9.2 Family Pterocladiaceae Felicini et Perrone 2006

Description: The thallus consists of prostrate and erect axes growing uniaxially; erect axes compressed to flattened, sparsely or pinnately branched; prostrate system consisting of terete to compressed stolons irregularly branched and attached by complex peg-like haptera consisting of internal rhizoidal filaments originated from inernal cortical cells, and multicellulare unisriate filaments originating from the surface cells. Triphasic isomorphic life history. Sexual plants dioecious or monoecious; unilocular cystocarps. Carposporangia developing on either one or all sides. Tetrasporangia in apical sori, arranged irregularly or in chevron–like rows (Perrone *et al*., 2006).

2.9.2.1 *Aphanta* Tronchin et Freshwater 2007

Description: The plants arise from robust, stoloniferous holdfast; uprights axes flattened. Branching distichous irregular to pinnate, opposite to subopposite, up to three orders; branches lanceolate to ligulate, basallyc onstricted. Flattened upright branches sometimes anastomose to one another and to the stolon; stolon terete often longer than the upright axes which develop at irregular intervals along its length. Nodes commonly found on stolon resulting from regrowth after injury; prostrate branches from the stolon produces brush-type attachment haptera. Only one species, *Aphanta pachyrriza* Tronchin et Freshwater, from South Africa was reported for the genus.

Aphanta was established based on molecular analyses of *rbc*L, SSU and LSU genes sequences and grouped in the order Gelidiales (Tronchin and Freshwater, 2007), although it possessed morphological characters of both families Gelidiaceae and Pterocladiaceae. Phylogenetic analyses of *rbc*L and SSU sequences did not show monophyly with neither Gelidiaceae nor Pterocladiaceae. But LSU sequences analyses showed closer relationship with Pterocladiaceae (Tronchin and Freshwater, 2007).

2.9.2.2 *Pterocladia* J. Agardh 1851

Description: The thallus is cartilaginous, sometimes crispate, composed of one or several erect axes, terete or compressed, distichously or irregularly branched. Erect axes arise from cylindrical or compressed, branched or unbranched creeping axes with numerous short haptera extending as individual axes or forming massive disc-like holdfasts. Elongated, colorless rhizines located in the medullary and/or cortical tissue. Sometimes varying in number and position within a given species. The tetraspores in the sori occupy expanded or broadly rounded tips of lateral branches or main axes.

Sporophylls can be single or pinnately compound. Tetrasporangia are cruciately divided, generally arranged without order in the sori although a few species may exhibit tetrasporangia borne in regular V-shaped rows. Spermatangial sori are sometimes apparent as relatively unpigmented areas on the apices of branchlets. The mature cystocarps are unilocular, protunding on only one of the surfaces of the branches, usually with one or more opening on only one surface of the frond. Carposporangia are usually formed in short chains. The genus is widespread in intertidal and shallow subtidal habitats of temperate and tropical waters. Absent from Arctic and Antarctic waters.

Historically 38 species have been reported in the genus, but evidences of unequal bilocular cystocarps in some species of the genus resulted in establishment of the genus *Pterocladiella* Santelices & Hommersand (Santelices and Hommersand, 1997) and except five species (Appendix 9), most *Pterocladia* species have been transferred to *Pterocladiella* (Appendix 10*)*, while some species were placed in genus *Ptilophora* (Appendix 11) and *Gelidium* (Appendix 12).

Nelson *et al.* (2006) found that *Pterocladiastrum*, based on the type species from the North Island of New Zealand, cannot be distinguished from *Pterocladia lucida* (Turner) J. Agardh using morphological and molecular analyses.

2.9.2.3 *Pterocladiella* Santelices et Hommersand 1997

Description: the plants has erect axes compressed to flattened, branching sparse to regularly or irregularly pinnate, attached to rocks by branched prostrate axes. Cortical

and medullary cells similar in size and arrangement to those of *Gelidium* species; rhizines in some specimens more numerous in medulla. Tetrasporangial sori terminaly, located on branches tips. Plants monoecious or dioecios, gametangial plants rare, spermatangia within apical sori. Cystocarps unilocular (some times with two unequal locules because of secondary detachment from the floor of cystocarp), carposporangia produce in chains from three sides of gonimoblast (Abbott, 1999).

Pterocladiella was separated from *Pterocladia* based on female reproductive structures. In the type species of the Pterocladiaceae, *Pterocladia lucida* (Brown ex Turner) J. Agardh, cystocarp is unilocular and the sporophyle is attached to the base of the cystocarp cavity. The evidences of unequal bilocular cystocarp in some species of *Pterocladia* such as *P. capillacea* (S.G. Gmelin) Bornet (Fan, 1961; Hommersand and Fredericq, 1988, Santelices, 1990) and detailed studies on cystocarp structure of different species of Gelidiales (Santelices, 1991, Hommersand and Fredericq 1996) resulted in establishment of the genus *Pterocladiella* (Santelices and Hommersand, 1997) to accommodate those species of *Pterocladia* which have unequal bilocular cystocarps with central placenta; their gonimoblast orginating from second order of nutritive filament cells in the core of the placenta. Several studies on the reproductive and vegetative characteristics of the Gelidiales (Santelices, 1998, 1999; Santelices and Hommersand, 1997) supported the establishment and validity of *Pterocladiella*, to which most species of *Pterocladia* and some species of *Gelidiella* and *Gelidium* were, transferred (Santelices and Hommersand, 1997; Santelices, 1998, 2007). In recent years, various studies using a combination of morphological and molecular data have supported the monophyly of *Pterocladiella* (Santelices and Hommersand, 1997; Freshwater and Bailey, 1998; Shimada and Masuda, 2000, 2002; Thomas and Freshwater, 2001; Millar and Freshwater, 2005; Nelson *et al.*, 2006; Tronchin and

Freshwater, 2007; Freshwater *et al.*, 2010). The lists of currently accepted species of *Pterocladiella* is shown in Appendix 12.

2.9.3. Family Gelidiellaceae Fan 1961

Description: Thalli consisting of prostrate and erect axes growing uniaxially; with main stolon serving as the main axis. Erect branches are terete to flattened, sparsely or pinnately branched. Thallus devoid of internal thick-walled rhizines. Subapical cells distichously or decussately dividing. Thallus attachment to substratum by independent unicellular rhizoidal filaments. Female reproductive system unknown. Spermatangial sori in apices of main axes or lateral branchlets. Tetrasporangial sori in conical or compressed apical regions of main axes and laterals; tetrasporangia arranged irregularly or in parallel transverse or chevron-like rows; tetrasporangia tetrahedrally or decussately divided (Perrone *et al.*, 2006).

2.9.3.1 *Gelidiella* Feldmann & G. Hamel 1934

Description: Thallus is formed by several tufted, entangled, cylindrical erect axes arising from decumbent and sometimes arcuate creeping axes, attached to the substratum by rhizoids. Erect axes cylindrical or slightly compressed, sometimes gradually tapering towards the apices and usually with sparse, filiform, distichously arranged, opposite or subopposite pinnae. Rhizines are absent from cortex and medulla. Tetrasporangial sori terminally, on the vetative pinnae, generally modified into onical swollen branches. Tetrasporangia ovoid, tetrahedric, cruciately or irregularly divided; may or may not be regularly disposed in the sore. Cystocarps unknown (Guiry and

Guiry, 2012). From 30 species of *Gelidiella* historically reported in this genus, currently 17 species are accepted as *Gelidiella* (Appendix 13), three species have transferred to the genus *Pterocladiella* (Appendix 14) and eight species to *Parviphycus* (Appendix 15), one species to Gelidium and one has been synonyme with *G. acerosa*.

2.9.3.2 *Parviphycus* Santelices 2004

Description: Thallus composed of upright branches arising from prostrate branches. Prostrate axis with indeterminate growth. The sub-apical cell with longitudinal division in two directions and subdistichous pattern. Creeping axes rounded or slightly compressed, attached to the substratum by unicellular or few-celled rhizoids, originating from the outer cortical cells, developing separately one from the other, forming a fringe of contiguous or bundle of isolated rhizoids. Upright branches normally arise from the face of the axis opposing the fringe of rhizoids. In cross section, the erect axes formed by 1 to 3 layers of cortical cells and 1 to 5 layers of medullary cells arranged in transverse rows. These are the axial cells flanked by one or several horizontally aligned periaxial cells on each side of axial cell. Tetrsporangia born on lanceolate, globose, cylindrical or terete stichidia located on short pedicels or in the apices of terminal branches; always one stichidium per pedicel or branch. Tetrasporangia formed in regular sequence from the apex by periaxial cells flanking the axial filament.

Currently eight species of *Parviphycus* are accepted in the Algaebase website (Appendix 15).

2.10 Gelidiales Studies in Southeast Asia

The first species of Gelidiales reported from Southeast Asia was *Porphyroglossum* Zollingeri recorded from Java, Indonesia (Kützing, 1847). Later studies reported Gelidiales species from Indonesia (Atmadja and Prud'homme van Reine, 2012; Silva *et al.,* 1996; Santelices, 2007; Hatta *et al.,* 1991; Verheij and Prud'homme van Reine, 1993), Malaysia (Santelices, 1997; Ahmad, 1995; Silva *et al.,* 1996, Phang *et al.,* 2007, 2008), Myanmar (Silva *et al.,* 1996), Philippines (Silva *et al.,* 1987, Lin and Freshwater, 2008; Trono 1973), Vietnam (Abbott *et al.,* 2002, Pham-Hoàng, 1969, Tsutsui *et al.,* 2005, Santelices, 2004), Singapore (Silva *et al.,* 1996; Lee *et al.,* 2009) and Thailand (Coppejans *et al.,* 2011, Tsutsui *et al.,* 2012, Santelices, 2004, Velasquez *et al.,* 1975; Wiriyadamrikul *et al.,* 2010). In Silva *et al*.'s (1996) monograph 26 species of Gelidiales were reported from Southeast Asia and recent studies on the seaweeds of the area (Thomas and Freshwater, 2001; Kraft *et al.,* 1999; Tronchin *et al.,* 2003; Santelices, 1997; Santelices and Flores 2004; Santelices, 2002, 2004; Phang, 1994, 1998, 2006; Phang *et al.,* 2007, 2008, Kim & Boo, 2011) has increased the number of Gelidiales species to 36, which their distribution in Southeast Asian countries has shown in Table 2.3.

Several studies have been carried out on seaweeds of Malaysia (Arumugam, 1981; Kawaguchi *et al.,* 2002; Phang and Wee, 1991; Ismail, 1995; Masuda *et al.,* 2001, 2002a, 2002b, Terada *et al.,* 2000; Phang, 1998, 2006; Phang *et al.,* 2007, 2008; Yamagishi *et al.,* 2003, Lim *et al.,* 2006) which reported a total of eight species of Gelidiales. Indonesia, with 21 species has the highest number of Gelidiales followed by Philippines (19 species), Vietnam (14 species), Malaysia (8 species), Singapore (3 species), Thailand (2 species) and Myanmar (1 species).

Five species of *Pterocladiella* have been reported from Southeast Asia with four in Philippines, three species reported from Indonesia and Vietnam and two species from Malaysia. Number of reported species of this order from neighboring countries like Indonesia, Philippines and Vietnam (Table 2.3) shows that diversity of the order in Malaysia should be more than previously reported, leading do the present study for this thesis.

Table 2.3: Gelidiales species and their distribution in Southeast Asia.

Species	IND	MY.	MYN.	PHL	SING	THI	VITt
Gelidiella acerosa (Forsskål) Feldmann & G. Hamel *****	X	X		X	X		X
G. bornetii (Weber-van Bosse) Feldmann & G. Hamel	X						
G. lubrica (Kützing) Feldmann & G. Hamel *****	X	X				X	X
G. fanii S.-M. Lin in Lin & Freshwater						X	
G. myrioclada (Børgesen) Feldmann & G. Hamel							X
Gelidium amansii (J.V. Lamouroux) J.V. Lamouroux *****		X		X	X		
G. capense (S.G. Gmelin) P.C. Silva	X						
G. amboniense Hatta & Prud'homme van Reine				X			
G. corneum var. *subrigidum* Grunow	X						
G. corneum (Hudson) J.V. Lamouroux	X		X				
G. crinale (Hare ex Turner) Gaillon	X			X			X
G. crinale var.*perpusillum* Piccone & Grunow	X			X			
G. coulteri Harvey				X			
G. indonesianum K.M.Kim, G.S.Gerung & S.M.Boo				X			X
G. divaricatum G. Martens				X			
G. isabelae W.R. Taylor				X			
G. kintaroi (Okamura) Yamada	X						
G. minusculum (Weber-van Bosse) R.E. Norris				X			X
G. pulchellum (Turner) Kützing				X			
G. pusillum var. pacificum W.R. Taylor	X	X	X	X	X		X
G. pusillum (Stackhouse) Le Jolis 1863*****				X			
G. rigens (C. Agardh) Greville ex Kützing							X
G. spathulatum (Kützing) Bornet	X	X					
G. spinosum (S. G. Gmelin) P. C. Silva*****	X						
G. spinosum f. *elongatum* (Hatta & Prud'homme van Reine) P. Silva							X
G. vietnamense Pham Hoang Ho	X						
G. zollingeri Sonder				X			X
Parviphycus pannosus (Feldmann) G.Furnari *****	X	X				X	X
P. adnatus (E.Y.Dawson) B.Santelices	X						
Pterocladia lucida (R.Brown ex Turner) J. Agardh	X						
Pterocladiella caerulescens (Kützing) Santlices & Hommersand *****	X	X					X
P. caloglossoides (M.A. Howe) Santelices *****	X	X		X			X
*P. capillacea (*S.G. Gmelin) Santelices & Hommersand				X			
Pterocladiella nana (Okamura) Shimada, Horiguchi & Masuda				X			
P. taylorii (Joly) Santelices	X			X			X
Ptilophora scalaramosa (Kraft) R.E. Norris	X			X			
Total	21	8	2	19	3	3	14

IND = Indonesia, MY = Malaysia, MYN = Myanmar, PHL = Philippines, SING = Singapore, THI = Thailand, VIT = Vietnamt (* sign shows the species reported from from Malaysia)

3.0 MATERIALS AND METHODS

3.1 Collection of specimens

The seaweed samples were collected from various localities along the coastline of Peninsular Malaysia during low tide and by snorkeling. Localities of collected samples are presented in Figure 3.1(see Appendix 16 for information of localities). Most of the Malaysian specimens were very small and collected by scraping from the rocks and coral surfaces. The collected samples were cleaned carefully under a stereomicroscope to remove dirt and epiphytes. The cleaned plants were sorted based on morphological similarities of their vegetative and reproductive structures.

3.2 Morphological studies

The sorted specimen was again cleaned carefully and epiphytes were removed by brushing and the cleaned samples were processed as follows:

i. Pressed onto herbarium sheets to preserve as voucher specimens deposited at the University of Malaya Seaweed and Seagrasses Herbarium.

ii. Some complete samples of each specimen were mounted on slides with Karo corn syrup and dried in 38-42℃ and used for morphological studies.

iii. Some cleaned samples of each specimen were fixed in formalin solution (3-5% formalin/seawater) for morphological and anatomical studies.

iv. Plants that were thoroughly cleaned of all epiphytes and contaminants were washed with distilled water and dried using C-Fold towel and then dried in silica gel and stored in -20℃ for molecular analyses.

Figure 3.1: Map of sample collection sites. modified from google online map site: http://maps.google.com.my/maps) : (1)Port Dickson (three stations), (2) Panti Dickson, (3) Pulau Besar, Malaka, (4) Pantai Cherating , Kelang, (5) Pulau Pinang (four stations), (6)Kuching, Sarawak (two stations) (7) Manukan island, Kota Kinabau (8)Pulau Nanuyang Laut, Sandakan (9) Kampung Dandulite, Sabah,(10) Kuala Terengganu (four stations).

Morphological and anatomical studies were conducted on fresh, fixed and herbarium specimens of collected samples. Because of the small sizes of samples, hand sections were made with a razor blade from fresh and fixed plants. The sections, if necessary, were stained in acidified aniline blue and mounted in 50% Karo corn syrup. The prepared slides were studied using stereomicroscope (Olympus XZH-10) and light microscope (Olympus BH-2) and photographed by Olympus Digital Camera.

For morphological studies many features of each specimen were studied as follows:

3.2.1 Vegetative features

- ✓ Height and color of plants
- ✓ Diameter, width and thickness of prostrate and erect axes.
- ✓ Branching pattern, constriction of branch base and form of axes and branches.
- ✓ Shape, arrangement, and size of cortical cells in surface view of erect and prostrate branches.
- ✓ Shape, arrangement and size of cortical cell layers, medulla and rhizines in transverse and longitudinal section of erect and prostrate stolon.
- ✓ Form, diameter and height of rhizoids.
- ✓ Form and position of apical cells.

3.2.2 Reproductive structures

- ✓ Form, location, arrangement and size of tetrasporangial sori and tetrasporangia in surface view and transverse section.
- ✓ Form, location and size of female reproductive structures in surface view and cross section.
- ✓ Form, location, arrangement and size of spermatangial sori in male stichidia.

3.3 Molecular Studies

3.3.1 DNA extraction and molecular studies

Total genomic DNA was extracted using the DNeasy Plant Mini Kit (Qiagen, Germany) according to the manufacturer's protocols. For extraction of total genomic DNA about 20 mg of silica gel dried samples of each specimen were first pulverized using liquid nitrogen and then lysed by adding lysis buffer and RNAase and incubation at 65˚C. After the lyses process, precipitation of protein and polysaccharides was carried out by salt precipitation. The precipitated debris was separated by centrifuging and transferring of supernatant in QIAshredder column. The column was spun in a microcentrifuge and the clear lysate was transferred to a new tube. Binding buffer and ethanol was added to bind DNA to the DNeasy membrane by a brief spin. The bound DNA on the membrane was washed and then eluted in 100µl of TE buffer by brief spin and stored in -20˚C.

The extracted DNA of each specimen was subjected to PCR amplification to amplify the partial sequences of the following genes:

1. *rbc*L gene (ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit)
2. *cox*I (mitochondrial-encoded cytochrome oxidase subunit I)
3. LSU (nuclear- encoded large subunit ribosomal RNA gene).

Four sets of published primers including F7 and Rbcs start, F57 and Rbcs start, F7 and R851, F645 and Rbcs start, were used for *rbc*L partial sequences amplification (Table 3.1). For *cox*I gene partial sequences, paired sets of primers including COX143F and COX1 1549R, COX143F and C880R, C622F and COX1 1549F, were used for amplification (Table 3.1). For Partial LSU gene sequence the set primers T04 and T08 were used in PCR amplification (Table 3.1)

3.3.2 Polymerase chain reaction (PCR)

Polymerase chain reaction (PCR) amplification of all genes was carried out using a Multigene Thermal cycler TC 9600-G (Lab Net International Inc., USA). Total volume for the PCR amplification for *rbc*L gene was 20 µl and consisted of 2.0 µl of 10x i-Taq plus reaction buffer, 0.25 mM dNTP mixture, 10 µM of each primer, 1.25 units of i-Taq plus DNA polymerase (iNtRON Biotechnology, Seoul, Korea) and 25-50 ng of DNA and the final volume was adjusted to 20 µL with ultra high quality (UHQ) water.

Table 3.1: List of the Published primers sequences used for PCR amplification.

Primer name	Primer sequences	Reference
F7	5′ - AACTCTGTAGTAGAACGNACAAG-3′	Freshwater & Rueness 1994
F57	5′ - GTAATTCGATATGCTAAAATGGG-3′	Freshwater & Rueness 1994
F645	5’ - ATGCGTTGGAAAGAAAGATTCT-3′	Lin *et al.* 2001
R1381	5 ’- ATCTTTCCATAGATCTAAAGC- 3 ’	Freshwater & Rueness 1994
Rbcs-Start	5′- GTTCTTTGTGTTAATCTCAC-3′	Freshwater & Rueness 1994
COXI 43F	5′- TCAACAAATCATAAAGATATTGGWACT-3′	Geraldino *et al.*, 2006
C622F	5′– CCTGTNGCAGGWGCTATTACAATGC-3′	Yang *et al.*, 2008
C880R	5′– ACAGTATACATATGATGNGCTCAAAC-3′	Yang *et al.*, 2008
COXI 1549R	5′- AGGCATTTCTTCAAANGTATGATA-3′	Geraldino *et al.*, 2006
T04	5′- GCAGGACGGTGGCCATGGAAGT-3′	Harper & Saunders 2001
T08	5′- CAGAGCACTGGGCAGAAATCAC-3′	Freshwater & Bailey 1995

The parameters of PCR amplification for rbcL gene were set with an initial denaturation cycle for 10 min at 94°C, followed by 35 cycles at 94°C for 30 sec, annealing at 48°C for 30 sec, extension at 72°C for 2 min. and a final extension at 72°C for 10 min for all primers (Table 3.2).

For *cox*I gene amplification, parameters of PCR amplification were set with an initial denaturation cycle for 10 min at 94°C, followed by 35 cycles of amplification

(denaturation at 90°C for 30 sec, annealing at 50°C for 45sec, and extension at 72°C for 7 min) with a final extension at 72°C for 10 min for all primers (Table 3. 2).

For LSU gene amplification, PCR amplification parameters were set the initial denaturation cycle for 3 min at 94°C, followed by 30 cycles of amplification (denaturation at 94°C for 30 sec, annealing at 50°C for 30 sec, and extension at 72°C for 45 min) with a final extension at 72°C for 5 min. The PCR products were viewed by electrophoresis on agarose gel 1% (W/v), stained by Sybr safe (SYBR® Safe DNA Gel Stain, Life Technology) (0.2mg mL^{-1}) and viewed under UV by Alpha imager.

Table 3.2: PCR program protocols used for *rbc*L, *cox*I and LSU genes amplifying

*rbc*L gene amplification protocol				
1 cycle	35 cycle			1cycle
94℃	94℃	48℃	72℃	72℃
10 min.	30 sec.	30 sec.	2min	10min.
*cox*I gene amplification protocol				
1 cycle	35 cycle			1cycle
94℃	90℃	50℃	72℃	72℃
10 min.	30sec.	45sec.	7min.	10min
LSU gene amplification protocol				
1 cycle	30 cycle			1cycle
94℃	94℃	50℃	72℃	72℃
3min	30sec.	30sec.	45sec.	5min.

3.3.3 Spectrophotometric Determination

The purity of the extracted DNA can be estimated by calculating the ratio between the optical density measured at 260 nm and 280nm (OD_{260}/OD_{280}). For the pure DNA, the ratio should be 1.8-2.0. Contamination with protein or phenol significantly decreases the above ratio. An OD of 1 at 260nm is approximately equivalent to 50µg ML $^{-1}$ of double strand DNA (dsDNA).

The DNA concentration for unknown sample = OD_{260} x 50µg ML $^{-1}$

The PCR products were further purified using PCR purification Kits (Qiagen, Germany) and sent to 1stBase (Malaysia) for sequencing using the primers of the initial PCR reactions.

3.3.4 PCR Products Purification and DNA Sequencing

The PCR products were purified using PCR purifying kits (Qiagen, Hilden, Germany) and sent to 1stBase (Kuala Lumpur, Malaysia) for sequencing using the primers of the initial PCR reactions

3.3.5 Phylogenetic analyses

The raw DNA sequences were edited using ChromasPro ver. 1.5 (Technelysium Pty Ltd, Australia). In addition to sequences obtained in this study, *rbc*L, *cox*I and LSU genes sequences of the Gelidiales species were acquired from GenBank (Appendices 17-19) and were included in the phylogenetic analysis. Three sets of sequences (*rbc*L, *cox*1 and LSU gene) were used for analyses of the species belong to family Pterocladiaceae to clarify the phylogenetic relationships between the species of this

family. For two families Gelidiaceae and Gelidiellaceae, two set of genes (*rbc*L & *cox*I) were analyzed separately for each family.

To determine the interfamilial relationship among the Gelidiales, the gene sequences of *rbc*L, *cox*I and LSU of the three families were acquired from GenBank together with sequences obtained from this study were concatenated for phylogenetic analyses.

The sequences were aligned initially using ClustalX n.2.0.8 (Larkin *et al.*, 2007), and subsequently manually aligned in BioEdit v.7.0.9.0 (Hall, 1999). The large gaps in LSU sequences alignment were assumed to be missing. Kakusan V.3 (Tanabe, 2007) was used to select the best fit model for (i) maximum likelihood (ML) and (ii) Bayesian Inference (BI) analyses. The best fit models were evaluated using the corrected Akaike Information Criterion (AICc; Akaike, 1973, 1978) for ML and the Bayesian information Criterion (BIC) for BI (Huelsenbeck and Ronquist, 2001).

ML analysis was performed with 1,000 bootstrap replicates via TreeFinder (Jobb *et al*., 2004; October 2008 version). BI analyses were performed using the Markov Chain Monte Carlo Method (MCMC) for 2×10^6 generations and sampling of the data every 100 generations. The likelihood scores stabilized after 200,000 generations. However, for our analyses a ‘‘burn-in’’ of 400,000 generations was used. To assess the level of variation in *rbc*L, *cox*I and LSU sequences pairwise genetic distances based on uncorrected model excluding gaps and ambiguities were simulated using PAUP 4.0b.10 (Swofford, 2002). Maximum Parsimony (MP) analyses were carried out with PAUP version 4.0b.10 and the trees were constructed using a heuristic search algorithm with 1000 random sequence additions, TBR branch swapping, with unordered and

unweighted characters. The bootstrap values were evaluated using 1000 bootstrapping replicates.

For interfamilial relationships of the Gelidiales order, the mean of pairwise divergence were constructed using Mega 4.0 software. In the interfamilial phylogenetic analyses, for *rbc*L only ML and BI were carried as MP was rather time consuming. For *cox*I and LSU , ML, Mp and BI analyses were carried out.

4.0 RESULTS

4.1 Morphological Studies

A list of 124 collected specimens is presented in Appendix 16. Thirteen morphospecies of Gelidiales selected for this study were collected from ten collection sites in Peninsular Malaysia and eastern Malaysia and were identified based on morphology. Identification of 11 species of Gelidiales from the collected specimens included two new species and two new records of the genus *Pterocladiella,* two new species and one new combination of the genus *Gelidium,* one species of the genus *Aphanta,* verification of two previously reported species, and identification of three morphological forms of the genus *Parviphycus.* Morphological comparison of identified species with other species of three families Pterocladiaceae, Gelidiaceae and Gelidiellaceae are shown in Tables 4.1, 4.2 and 4.3 and 4.4 respectively. Recognized and identified species in this study are as follows:

1. *P. bartlettii* (W.R.Taylor) Santelices
2. *P. beachiae* Freshwater in Thomas & Freshwater
3. *P. caerulescens* (Kützing) Santelices & Hommersand
4. *Pterocladiella* sp. nov.1
5. *Pterocladiella* sp. nov.2
6. *Aphanta sp*. nov.
7. *Gelidium* cf. *crinale* var. *perpusillum*
8. *Gelidium* sp. nov.1
9. *Gelidium* sp. nov.2
10. *Gelidiella acerosa* (Forsskål) Feldmann & G.Hamel
11. *Parviphycus* sp.1
12. *Parviphycus* sp.2
13. *Parviphycus* sp.3

4.1.1 FAMILY PTEROCLADIACEAE

4.1.1.1 *Pterocladiella bartlettii* (Taylor) Santelices (Figure 4.1)

Ref: Santelices, 1998, p. 239-242, figure 1; *Pterocladia bartlettii* W.R.Taylor 1943, p. 156; pl. 4, Figure 2., Thomas & Freshwater, 2001, p. 345-346, Figures 13-15.

Description: The largest collected samples were up to 3 cm, blackish (collected from Port Dickson) (Fig. 4.1A) to pink in color (collected from Pulau Pinang) (Figs. 4.1B, 4.1D), bushy, with erect axes and many narrow long entangled branches (Fig. 4.1A). Plants attached to substratum by peg-like rhizoids with discoid haptera (Fig. 4.1C). Erect axes cylindrical to semi-compressed basally, fattened in middle and upper parts, 71-250 µm in diameter basally, up to 603 µm wide in middle and upper parts and up to 155 µm thick. Branching irregular, pinnately bilateral or polytrichous (Figs. 4.1A, 4.1B) and sometimes producing bilateral linear lanceolate branches from the expanded tetrasporangial sorus areas (Fig. 4.1D). Branches lanceolate with basal constriction in young tetrasporangial branchlets, tapering to acute tips with a dome-shaped apical cell (Fig. 4.1E).

Cortical cells in surface view of erect branches rounded to polygonal at basal part (Fig. 4.1F) and ovoid to conical (Fig. 4.1G), 7-17.5 x 4-9 µm at middle area. In transverse section, erect branches composed of 2-4 layers of cortical cells and large medullary cells. The outermost cortical layers consisted of small isodiametric rounded to periclinally elliptical cells, 4-10 x 4-9 µm. Inner cortical cells size became larger, 8-20 x 5-16 µm, gradually at basal part (Fig. 4.1H) or abruptly at upper flattened area (Fig. 4.1I). Medullary cells, 9-18 x 10-16 µm, were arranged transversely in one to three rows. Rhizines were few in number among medullary cells (Figs. 4.1H & 4.1I). Longitudinal section of erect branches, consisted of 2-4 layers of cortical cells and medulla, outermost cortical cells were rounded to elliptical and arranged horizontally,

inner larger cortical cells and medullary cells arranged vertically and filled with small discoid chloroplasts (Fig. 4.1J).

Stolons and prostrate branches were cylindrical to semicompressed, 84 -216 µm in diameter and 44–116 µm in thick, attached to substratum by peg-like rhizoids with discoid holdfast at attachment point, up to 198 µm in diameter and 488 µm in height (Fig. 4.1C). Cortical cells in surface view of stolon were polygonal, 7-20 x 6-14 µm, irregular to transversely arranged and filled by discoid chloroplasts (Fig. 4.1K). 3-4 layers of cortical cells were observed in transverse section of the stolons, outermost cortical cells 8-13 x 6-8 µm, arranged periclinally (Figs. 4.1L & 4.1M) and inner cortical cells inwardly became larger, 13- 25 x 11-19 µm, abruptly in older parts (Fig. 4.1L) and gradually in younger parts (Fig. 4.1M). Rhizines were not observed among medullary cells. In longitudinal section of the stolon, outermost cortical cells was arranged vertically and inner larger cortical cells were arranged horizontally (Fig. 4.1N). Tetrasporangial sori were produced on lateral and apical branches or intercalary on main and lateral branches (Figs. 4.1O- 4.1Q), 223-1921 x 86-320 µm and up to 127µm thick; tetrasporangia were small, 10-37 x 6-25 µm and scattered or disposed regularly in V-shaped arrangement. Gametophyte plant was not observed.

Holotype locality: Cahuita, Limon, Costa Rica (Thomas & Freshwater 2001: 346).

Global distribution: North America (Texas), Caribbean Islands (Barbados, Cuba Haiti, Hispaniola, Jamaica), Western Atlantic, South America (Colombia Venezuela).

Local distribution in Malaysia: Port Dickson, Negeri Sembilan, (2° 24' 54" N / 101 ° 51' 10 " E), 27 Apr. 2011, J. Sohrabipoor, PSM12564 and 8.Jun 2012, PSM12664; Batu Feringhi 3, (5° 28' 51" N; 100 ° 15' 15" E) Pulau Pinang, 7 Sep. 2009, J. Sohrabipoor, PSM12495, PSM12496 and 8 Jun. 2012, PSM12664.

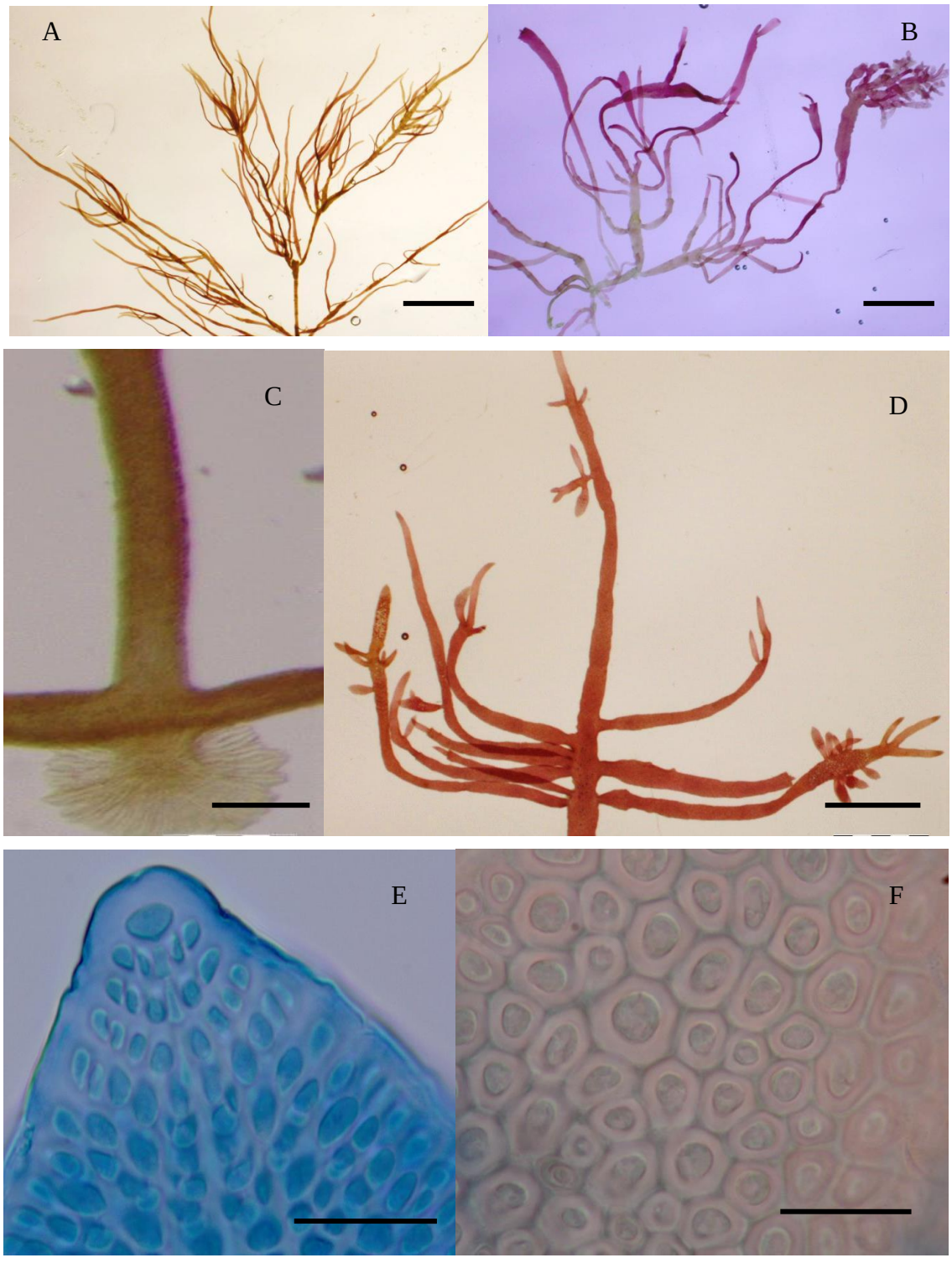

Figure 4.1: *Pterocladiella bartlettii* (Taylor) Santelices

(A) Habit of plant collected from Port Dickson. Scale bar = 2 mm, **(B)** Habit of plant collected from Pulau Pinang, Malaysia. Scale bar = 2 mm, **(C)** Short rhizoid with discoid holdfast. Scale bar = 200 µm, **(D)** divaricate ascending branches on margins of tetrasporangial sorus. Scale bar = 2 mm, **(E)** Apical cell and longitudinal division of sub-apical cells. Scale bar = 20 µm, **(F)** Polygonal to rounded cortical cells in surface view of erect axis base. Scale bar = 20 µm.

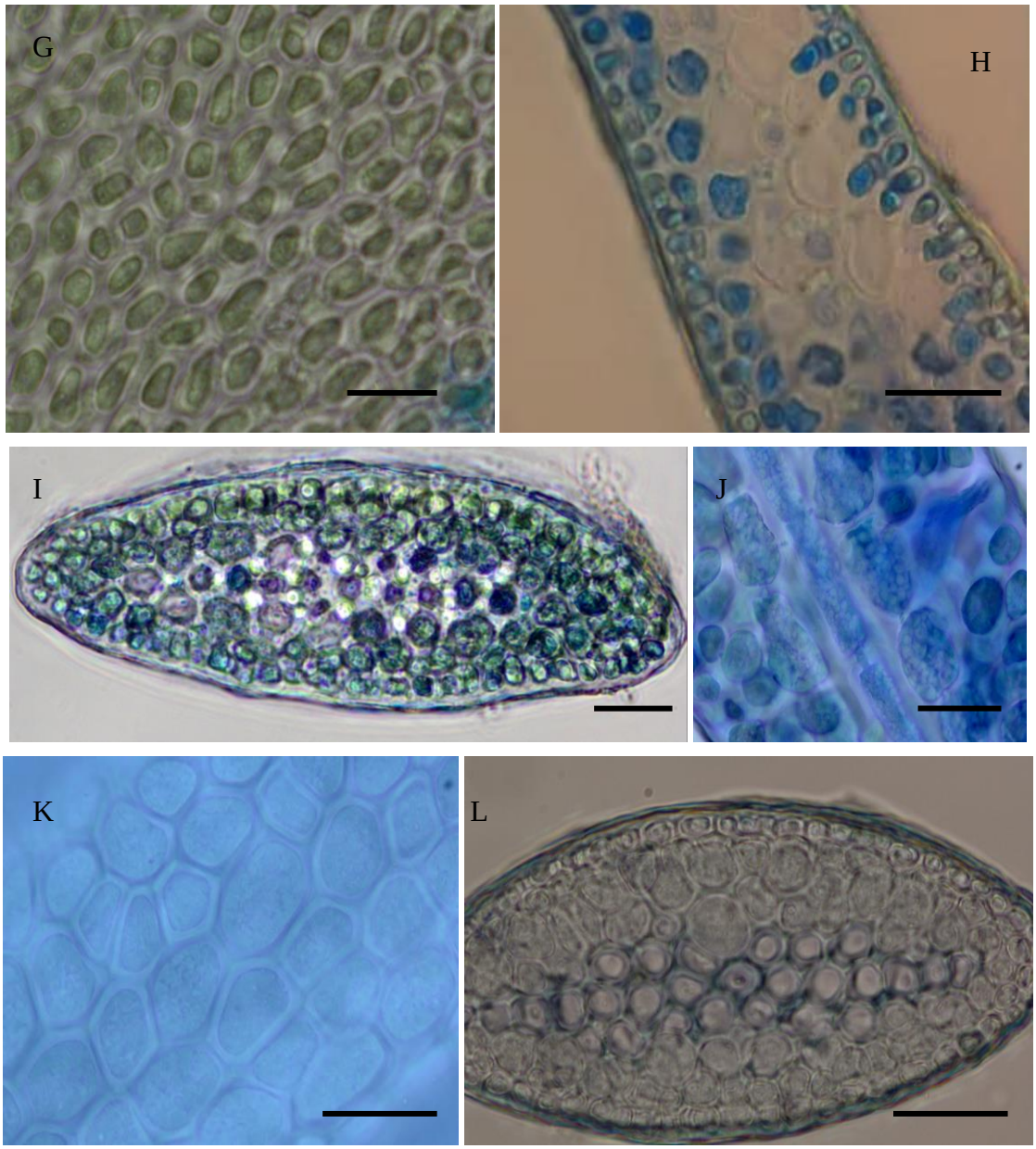

Figure 4.1: *Pterocladiella bartlettii* (Taylor) Santelices (continue)

(G) Long ovoid to conical cortical cells in surface view of erect axes. Scale bar = 20 µm, (H) Transverse section of erect axis shows 2-4 layers of cortical cells and 1-3 transverse rows of large medullary cells and low number of rhizines (arrows) . Scale bar = 40 µm (I) Transverse section of erect axes at lower semicompressed area shows 2-4 layers of cortical cells and rhizines (arrows) among medullary cells. Scale bar = 20 µm,, (J) Isodiametric cortical cells and vertical medullary cells in longitudinal section of erect axis. Scale bar = 20 µm, (K) Polygonal cortical cells arrangement on surface view of stolon. Scale bar = 20 µm, (L) Periclinal arrangement of outermost cortical cells, abruptly enlarging of inner cortical cells, 1-3 rows of smaller medullary cells and absence of rhizines in transverse section of older parts of stolon. Scale bar = 40 µm.

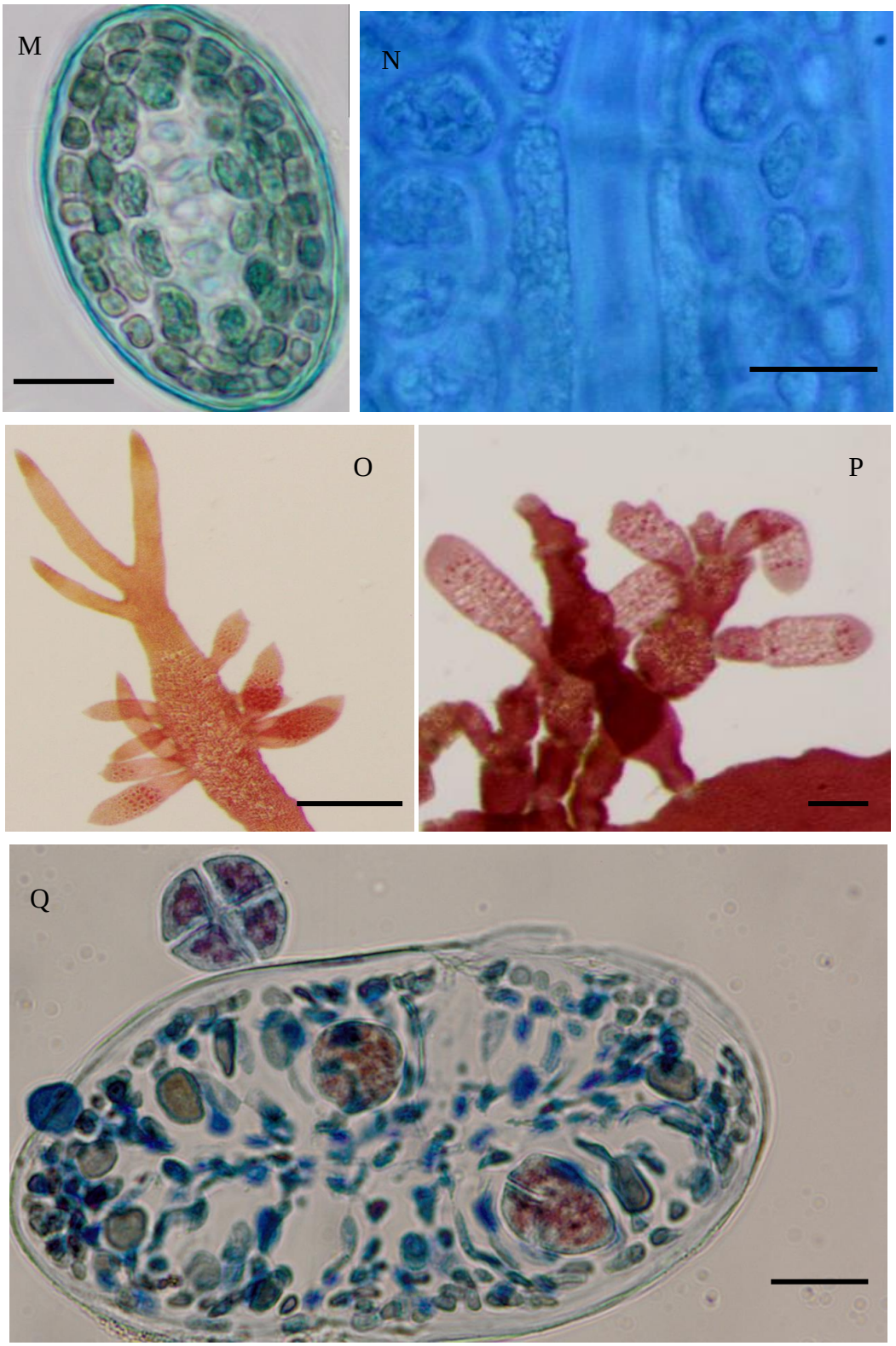

Figure 4.1: *Pterocladiella bartlettii* (Taylor) Santelices (continue)

(M) Periclinal arrangement of cortical cell layers, 1-3 rows of smaller medullary cells and absence of rhizines in transverse section of younger parts of stolon. Scale bar = 20 µm, **(N)** Vertical to horizontal arrangement of cortical cells in longitudinal section of stolon. Scale bar = 20 µm, **(O)** V-shaped tetrasporangial sorus with bilateral tetrasporangial branchlets at margins. Scale bar = 400 µm, **(P)** V-shaped terminal tetrasporangial sori. Scale bar = 200 µm, **(Q)** Transverse section of tetrasporangial sorus. Scale bar = 20 µm.

4.1.1.2 *Pterocladiella beachiae* Freshwater in Thomas & Freshwater (Figure 4.2)

Ref: Thomas & Freshwater, 2001, p. 340-350, (as *Pterocladiella beachii*); Millar & Freshwater, 2005, p. 252 (as *Pterocladiella beachiae* Thomas & Freshwater).

Description: Plants up to 3 cm tall, blackish red to green in color, consisting of erect axis and stolons. Erect axes lanceolate to ligulate, cylindrical at base and flattened at middle and upper parts (Figs. 4.2A-D), up to 1411 µm wide and 68 - 85 µm thick; branching sparse, alternate, distichous, irregular (Figs. 4.2B-C) to pinnate (Fig. 4.2D) and occasionally unbranched (Figs. 4.2A). Up to 3 orders of branching; branches are ligulate to lanceolate with constricted base and obtuse (Fig. 4.2C), emarginated to apiculate apices (Fig. 4.2D) with dome-shaped to spherical apical cells (Fig. 4.2E).

In surface view of erect axis, cells were conical, to oval shape, 5-13 x 2-5 µm and arranged parallel to axis of erect axes and branches (Fig. 4.2F). In transverse section, erect axis consisted of 2-4 layers of cortical cells and medulla (Fig. 4.2G). The outermost layer of cortical cells composed of quadrate to elliptical cells, 4-15 x 3-7 µm and arranged anticlinally. The inner cells were periclinally arranged (Fig. 4.2G), 8-12 x 5-8 µm; medullary cells were larger than cortical cells and arranged in long transverse rows. Translucent rhizines, 4-7.8 µm in diameter, scattered among medullary cells and sometimes surrounding the colorless thick walled medullary cells (Fig. 4.2G). Longitudinal section of the erect branches showed many layers of medullary cells, innermost long cortical cells, 28-32 µm in length, connected to the vertically arranged inner cortical cells, 10-20 x 4-6 µm, and outermost cortical cells, 6-14 x 3-7µm, obliquely connected to inner cortical cells (Fig. 4.2H).

Stolons semicompressed to terete, 150-358 µm in diameter and 123-205 µm thick, attached to substratum by peg-like rhizoids. Rhizoids 540-1050 µm in length and 176-283 µm in diameter.

In surface view in prostrate axes and stolons, cortical cells were oval to rounded, 7-15 x 3-8 µm, and mostly arranged parallel to stolon axis (Fig. 4.2I), In transverse section, stolon consisted of 3-4 layers of cortical cells and medulla, outermost cortical cells were elongated oval, 5-11 x 3-7 µm and arranged anticlinally whereas inner layers showed periclinal arrangement (Fig. 4.2J). Medulla comprising many cell layers (Fig. 4.2J -L) with outer long vertical cells in longitudinal section, filled with discoid chloroplasts (Fig. 4.2K); central layers of medulla composed of smaller cells and irregularly scattered rhizines (Fig. 4.2J).

Tetrasporangial sori were borne on subterminal parts of the main and lateral branches, occasionally series of 2-3 rounded sori were on longer branches (Fig. 4.2M). Mature sorus without sterile margin (Figs 4.2M-4.2O), protuberant and curved to adaxial sides of branches and branchlets, often terminated to a long and emarginated apex (Figs. 4.2N). Matured sori were 247-1075 x 237-931µm and 48-216 µm thick. Tetrasporangia disposed irregularly (4.2O), 12-21 µm in diameter and 19-43 x 9-21 µm in transverse section (Fig. 4.2P).

In carposporophyte plants, unequal bilocular cystocarps were observed in slender to oval or obovoid (Figs. 4.2Q-S) swollen forms, 367-1300 x 120-365 and 220-300 µm thick, on middle to sub-terminal parts of lateral and main branches and sometimes intercalary on main axes (Fig. 4.2S). Within the central cavity of the cystocarp a core of nutritive filaments produced gonimoblasts on the third order branches which originated from axial filaments (Fig. 4.2T). Carposporangia produced a large number of

carpospores released through an ostiole (Fig. 4.2T). Longitudinal sections through cystocarp showed the nutritive filaments connecting the carposporangial chain to cystocarp walls (Fig. 4.2U). Carpospores ovoid to conical in shape, 10-27 x 8-15 µm. Spermatangial sori in small or large patches located on margins and basal parts of cystocarps (Figs. 4.2V & 4.2W); spermatia have mean length/width ratio=2.9 .

Global distribution: Central America (Costa Rica, Panama)

Local distribution in Malaysia: Pulau Pinang (5º 28’ 51" N; E 100º 15’ 15"), 8 Sep. 2009, J. Sohrabipoor, PSM12497, 13. Feb.20l0, PSM12507; Pulau Besar, Melaka (N 2º 06' 56" E 102º 19' 54"), 11.Apr.2010, J. Sohrabipoor, PSM12519, PSM12520; Teluk Kemang , Negeri Sembilan (2º 26’ 38" N; 101º 51’ 21" E), 25 Nov. 2011, PSM12616; Port Dickson, Negeri Sembilan (2º 24 ' 54" N; 101º 51’20" E), 28 Feb. 2010, J. Sohrabipoor , PSM12515, PSM12516; Teluk Kemang, Negeri Sembilan (2º 26’ 38" N; E 101º 51’ 21"), 12 Jul. 2010, J. Sohrabipoor , PSM12538.

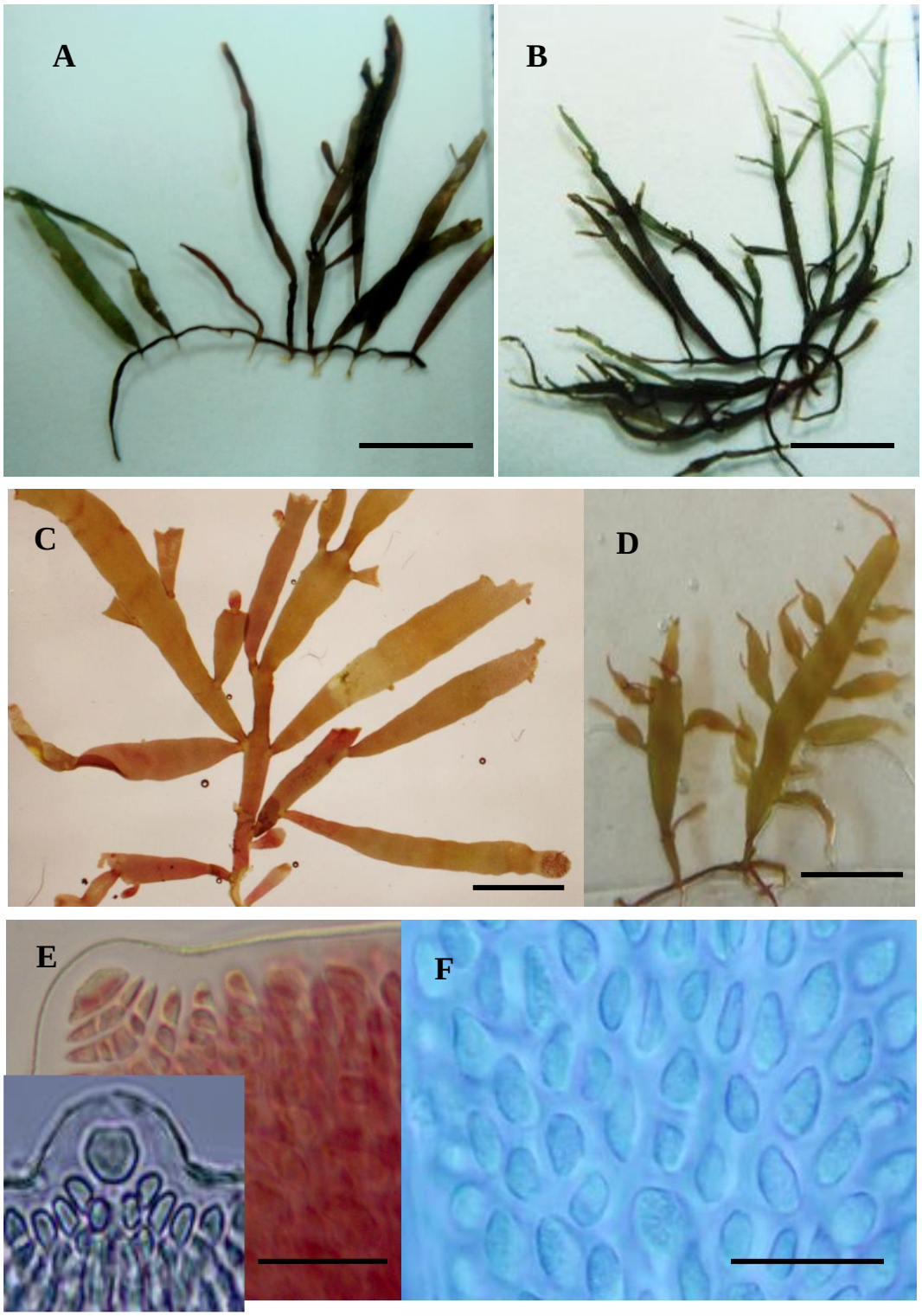

Figure 4.2: *Pterocladiella beachiae* Freshwater.

(A) Habit of plant, erect axis with rare lateral branches. Scale bar = 5 mm, **(B)** Habit of plant. Erect axis with sparsely irregular branching. Scale bar = 5 mm, **(C)** Habit of fresh tetrasporophyte with irregular branching. Scale bar = 2 mm, **(D)** Habit of cystocarpic plant with one order of pinnate branching. Scale bar =5 mm, **(E)** Dome-shaped and spherical apical cell and division pattern. Scale bar = 20 µm, **(F)** Conical cortical cells in surface view of erect axis. Scale bar = 20 µm.

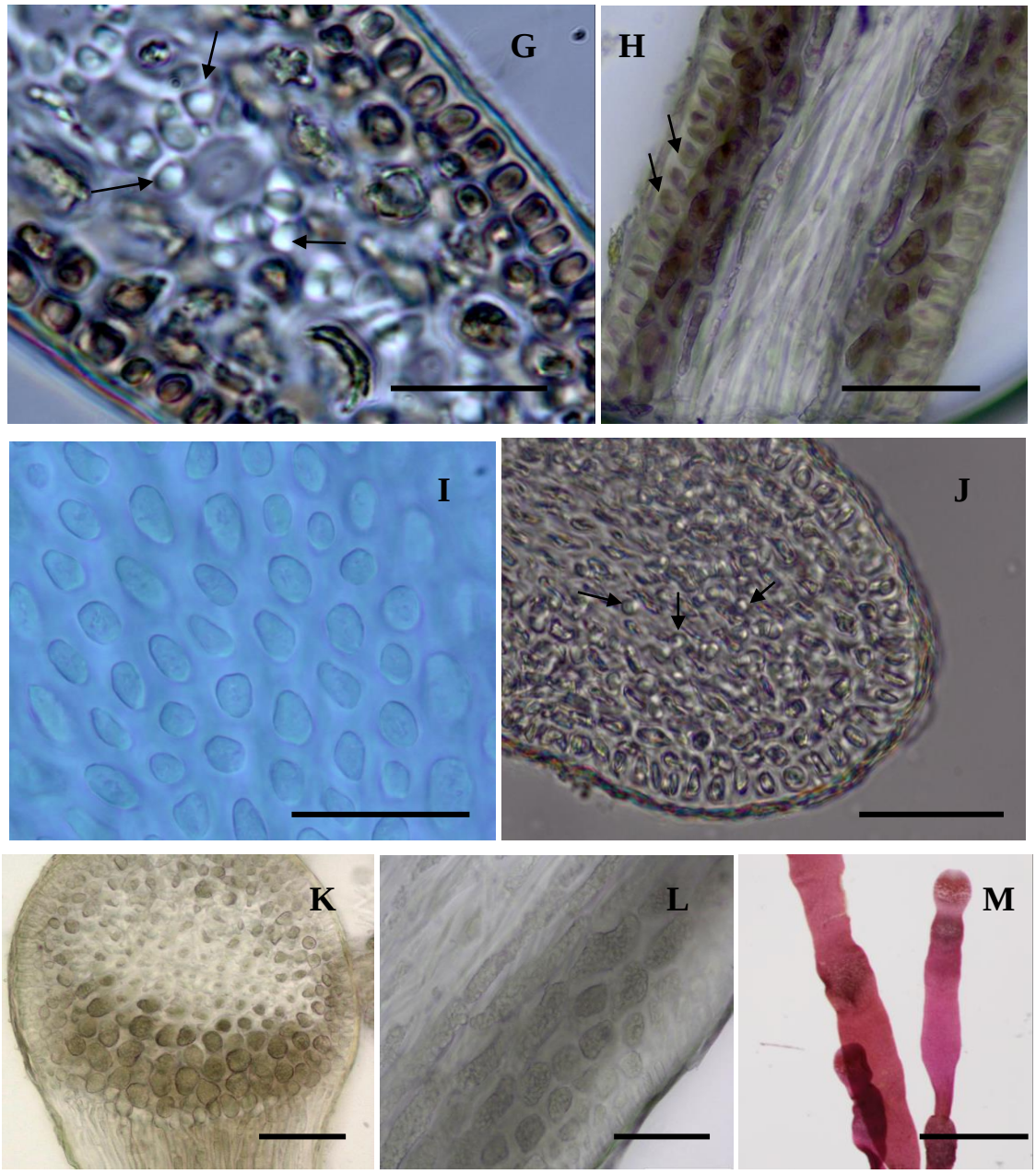

Figure 4.2: *Pterocladiella beachiae* Freshwater (continue)

(G) 2-4 layers of anticlinal outermost cortical cells in transverse section of erect axis, large medullary cells surrounded by rhizines (arrows). Scale bar = 20 µm, **(H)** Oblique arrangement of outermost cortical cells (arrows) and vertical arrangement of inner cortical and medullary cells in longitudinal section of erect axes. Scale bar = 20 µm, **(I)** Oval to rounded cortical cells in surface view of stolon. Scale bar = 20 µm, **(J)** Anticlinal arrangement of outermost cortical cells, isodiametric inner cells and low number of scattered rhizines in transverse section of stolon. Scale bar = 50 µm, **(K)** Rhizoidal filaments in peg-like rhizoids originated from inner cortical cells in longitudinal section of rhizoid. Scale bar = 50 µm, **(L)** Vertical arrangement of inner cortical and medullary cells and discoid chloroplast in cortical cells in longitudinal section of stolon. Scale bar = 50 µm, **(M)** Series of tetasporangial sori on main and lateral branches. Scale bar = 2 mm.

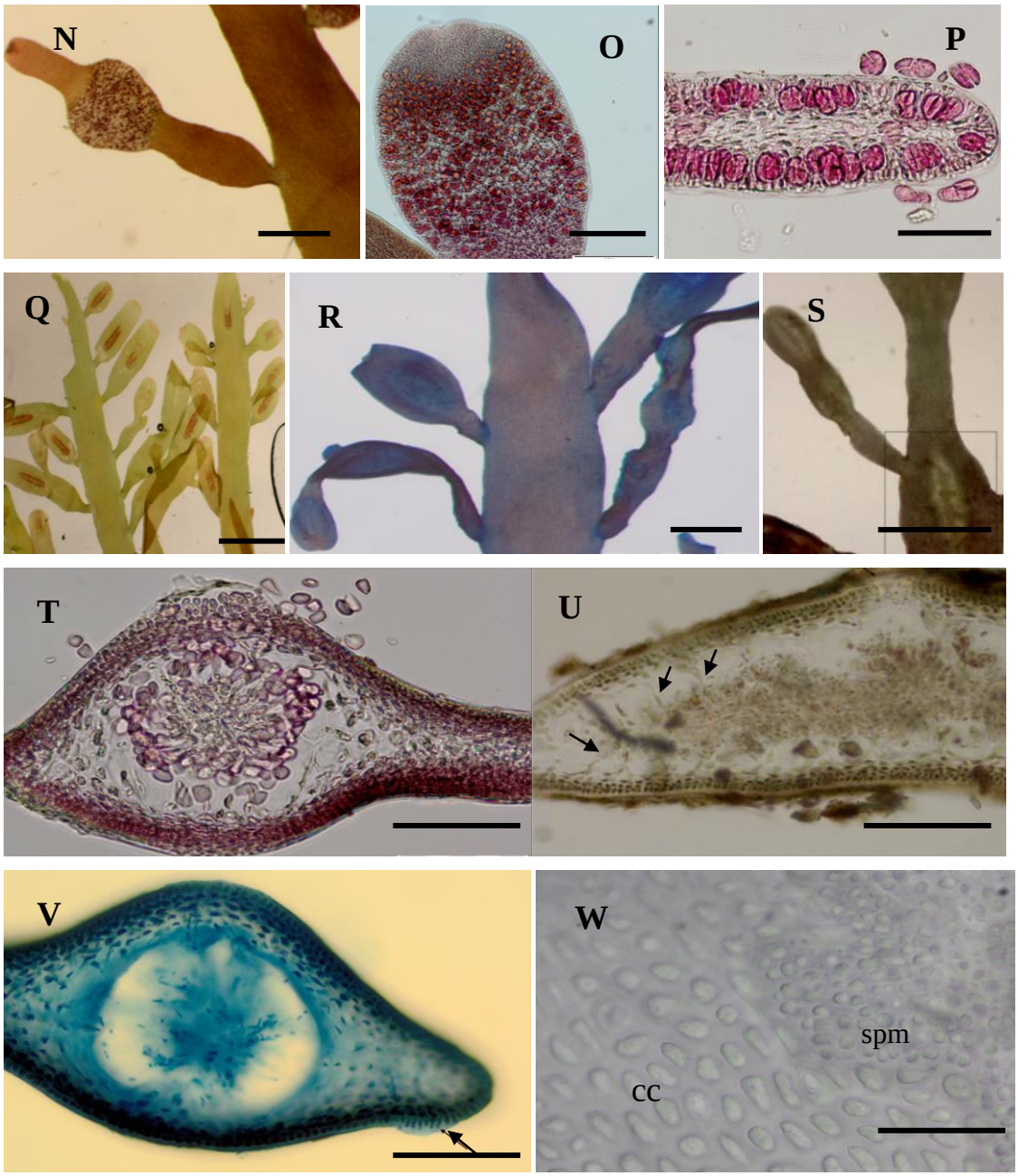

Figure 4.2: *Pterocladiella beachiae* Freshwater (continue)

(N) Irregularly arranged tetrasporangial sori with without sterile margin. Scale bar = 500m, **(O)** Irregular arrangement of tetrasporangia without sterile margin in surface view of tetrasporangial branchlets. Scale bar = 200µm, **(P)** Transverse section of tetrasporangial sorus shows large number of tetrasporangia. Scale bar = 100µm, **(Q)** Slender and swollen cystocarps on subterminal to middle parts of lateral branches and main axis. Scale bar = 2mm, **(R)** Oval shaped cystocarps with projected ostiole (arrow). Scale bar= 500µm, **(S)** Intercalary cystocarp on main axes. Scale bar = 500 µm, **(T)** Transverse section of cystocarp shows unequal bilocular form of cystocarp and central position of placenta core and large number of carpospores in all sides of placenta core. Scale bar =100 µm, **(U)** Longitudinal section of cystocarp shows a dense chain of placenta around central axis of cystocarp connected by nutritive filaments to the pericarp in both side (arrows), Scale bar = 100 µm, **(V)** Transvers section of cystocarp shows small patch of spermatangial sorus on margin of cystocarp (arrow). Scale bar= 100 µm, **(W)** A large patch of spermatangial sori (spm) among cortical cells (cc) of cystocarp (arrow), Scale bar = 20 µm.

4.1.1.3 *Pterocladiella caerulescens* (Kützing) Santelices et Hommersand (Figure 4.3)

Basionym: *Gelidium caerulescens* Kützing 1868, p.19, Pl. 56,

Synonyms: *Pterocladia caerulescens* (Kützing) Santelices, 1976. P.165-193; *Pterocladia tropica* Dawson, 1959, p.40, figs. 21A-d & 22B; *Gelidium irregularis* Loomis, 1960, p.6, Pl.9 & 10; *Pterocladia rigida* Loomis, 1960, p.8, Pl. 11;

Ref. Santelices, 1976. P.165-193 (as *Pterocladia caerulescens*); Santelices 1977, p.79, fig. 6F-G (as *Pterocladia caerulescens*), Santelices 1978, p. 53-59 (as *Pterocladia caerulescens*), Santelices & Hommersand 1997, p. 118; Xia *et al.*, 2004, pp.202-205, figs.9-16; Millar & Freshwater, 2005, p.251.

Description: Thalli up to 3 cm tall, blackish to green (Figs. 4.3A & 4.3B), erect and flattened axes arising from a terete prostrate stolon; erect axes cylindrical to semicompressed at base, 159 µm diameter, up to 1460 µm width at flattened upper parts and up to 160 µm thick; attached to substratum by peg-like rhizoidal holdfast . Erect axis branched densely to sparsely in irregular, alternately distichous to pinnate pattern, up to 3 orders (Figs 4.3A-C). Branches constricted at the base and terminating in obtuse, emarginated to apiculate tips (Fig. 4.3C) with dome-shaped apical cells (Fig. 4.3D). In surface view of erect axes and branches, cortical cells were oval to conical, 5-12 x 3-6 µm, which mostly were parallel to branch axis (Fig. 4.3E). In transverse section of erect axes, 3-4 layers of cortical cells were arranged anticlinally in outermost layer and periclinally in inner layers (Fig. 4.3F), cells in outermost layer oval to long oval, 5-10 x 3-6 µm and gradually became larger downward, 7-13 x 5-11 µm and filled with discoid chloroplasts. Abundant translucent small rhizines, 3-6 µm in diameter, surrounding the medullary cells (Fig. 4.3F). In longitudinal section of erect branches, outermost cortical cells oval to elliptical, 7.3-12 x 4.8-7 µm, arranged obliquely while cells of inner layers were vertically arranged (Fig. 4.3G).

Stolons cylindrical to semicompressed, 179-312 µm in diameter and 159-283 µm thick, attached to substratum with peg-like rhizoids (Fig. 4.3H); rhizoides 396-1086 µm

in height and 180-314 µm in diameter. In surface view of stolons, cortical cells were angular to conical, 7-14 x 4-10 µm, parallel to stolon axis or in irregular highly spaced arrangement (Fig. 4.3I). In transverse section, stolons consisted of three to four layers of cortical cells; outermost cells small, 7.4- 12.3 x 4.4-8.6µm, rounded to elongated oval-shaped and arranged anticlinally (Fig. 4.3J), inner cells became larger, 10.5-19 x 8.7-14µm, arranged periclinally and filled with discoid chloroplasts (Fig. 4.3J). Rhizines small, 4-5 µm in diameter and scattered among medullary cells (Fig. 4.3K). Longitudinal section of the stolon showed the outermost elliptical cells arranged obliquely while inner cells showed vertical arrangement (Fig. 4.3L).

Long-oval to rarely rounded, tetrasporangial sori with sterile margins were located on lateral branches and branchlets (Figs. 4.3M & 4.3N), 743-2185 x 300-667µm and 100-140 µm thick, irregularly arranged tetrasporangia were 18-25 µm diameter, in surface view (Fig. 4.3O) and 31-41 x 20-27 µm in transverse section (Fig. 4.3P).

Cystocarps were formed as elongate cylindrical swellings with two unequal locules on lateral branches and subterminal area of main axes (Fig. 4.3Q); 700-1382 x 148-205 µm and 200 - 220 µm thick, with a wide sterile margin, up to 240 µm width which is equally disposed on two sides of the cystocarp. In transverse and longituidinal sections of cystocarps, central core consisted of nutritive filaments, gonimoblast and carposporangia (Fig. 4.3R-T). Filaments of gonimoblast originated from third-order cells derived from nutritive filaments of axial filament. In matured cystocarp, the cylindrical chain of placenta connected to cystocarp walls on all sides and pericarp with single ostiole expanded to adaxial sides of branches (Figs. 4.3R-T). Carpospores dimension were 17-26 x 9-17 µm. Spermatangial sori were observed in small and large patches on cystocarp wall and basal parts of cystocarp (Figs. 4.3U & 4.3V). Spermatia

small, 3-4.4 x 1.9-2.8 µm, cut off by transverse division of spermatangial initial cells (Fig. 4.3W).

Type locality: Wagap, New Caledonia (Millar & Freshwater 2005: 252).

Global distribution: Atlantic Islands (Bermuda), Pacific Islands (New Caledonia, Hawaiian Islands, French Polynesia, Fiji), Caribbean Islands (Barbados, Trinidad, Tobago), South America (Brazil, Venezuela, Colombia), Africa (Ethiopia, Eritrea, Morocco, South Africa), Asia (China, Korea, Japan), Indian Ocean Islands, South-west Asia (India, Oman, Sri Lanka), Southeast Asia (Indonesia, Malaysia, Vietnam), Australia, Central America (Panama).

Local distribution in Malaysia: Port Dickson, Negeri Sembilan (2° 24' 54" N; 101° 51'10" E), 30 Dec. 2009, J. Sohrabipoor, PSM12501; Port Dickson (2 ° 24' 54" N; 101 ° 51' 10" E), 30 Dec. 2009, J. Sohrabipoor, 10 Jun. 2010, J. Sohrabipoor PSM12530, PSM12531, PSM12526; Teluk Kemang, Negeri Sembilan, (2º 26’ 38" N; 101º 51’ 21" E), 12 Jul. 2010, J. Sohrabipoor, PSM12537; Teluk Kemang , Negeri Sembilan (2º 26’ 38" N; 101º 51’ 21" E), J. Sohrabipoor, PSM12662; Pulau Nunuyang Laut, Sandakan, Sabah (5 ° 55' 24" N; 118 ° 05' 28" E), 28 Nov. 2010, J. Sohrabipoor, PSM546.

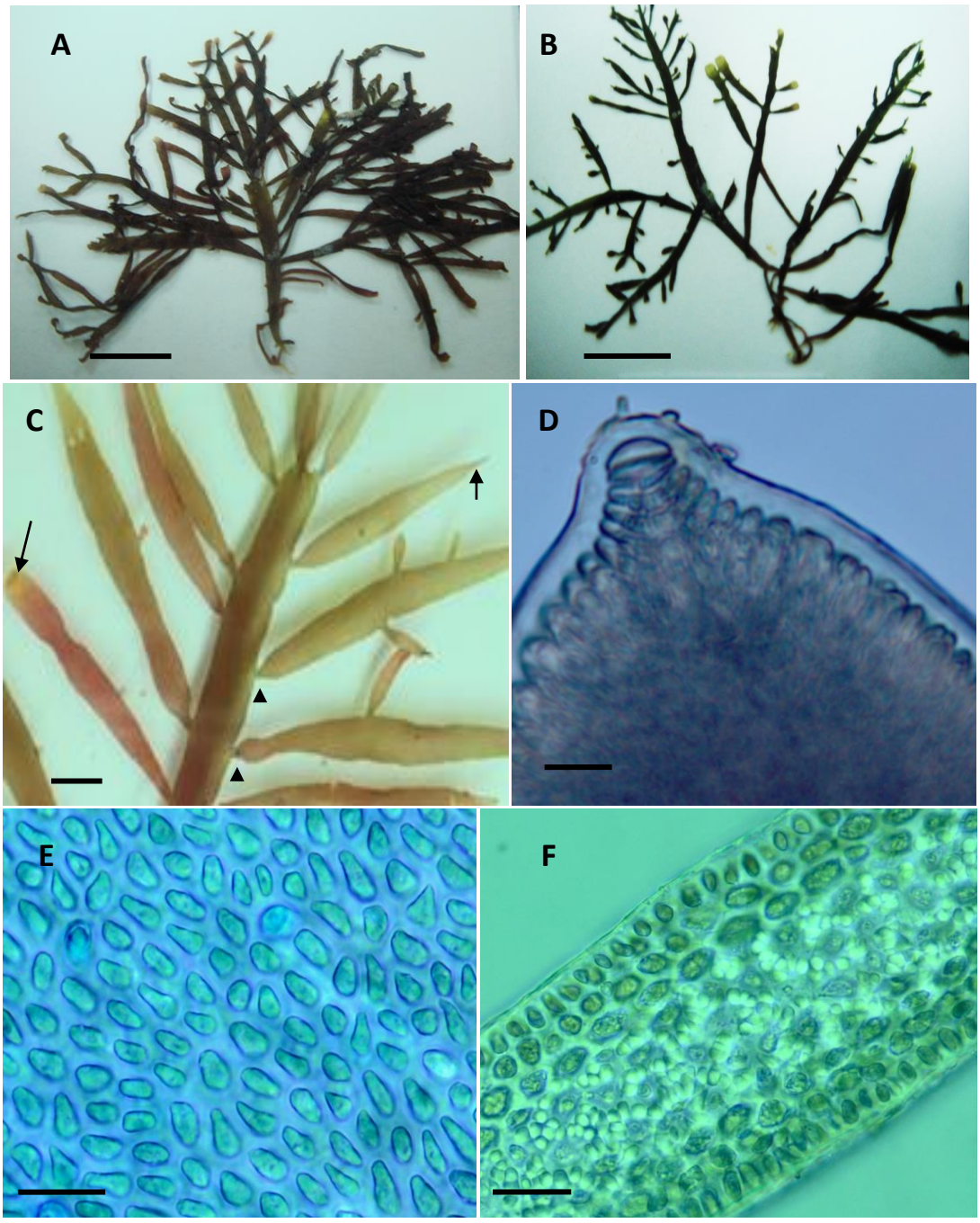

Figure 4.3: *Pterocladiella caerulescens* (Kützing) Santelices et Hommersand.

(A) Habit of plant, with densely opposite branching of axis (PSM12530). Scale bar = 5mm, (B) Habit of plant with sparse and irregular branching of axis (PSM12501). Scale bar=5 mm, (C) Opposite to alternate branching pattern and basal constriction (arrow head) with emarginated (long arrow) and apiculate apices (short arrow). Scale bar = 2 mm, (D) Dome-shaped apical cell. Scale bar = 10 µm, (E) Ovoid to conical cortical cells in surface view of erect axis. Scale bar = 20 µm, (F) Anticlinal outermost cortical cells, periclinal inner cells and rhizines in medull in transverse section of erect axis. Scale bar = 20 µm.

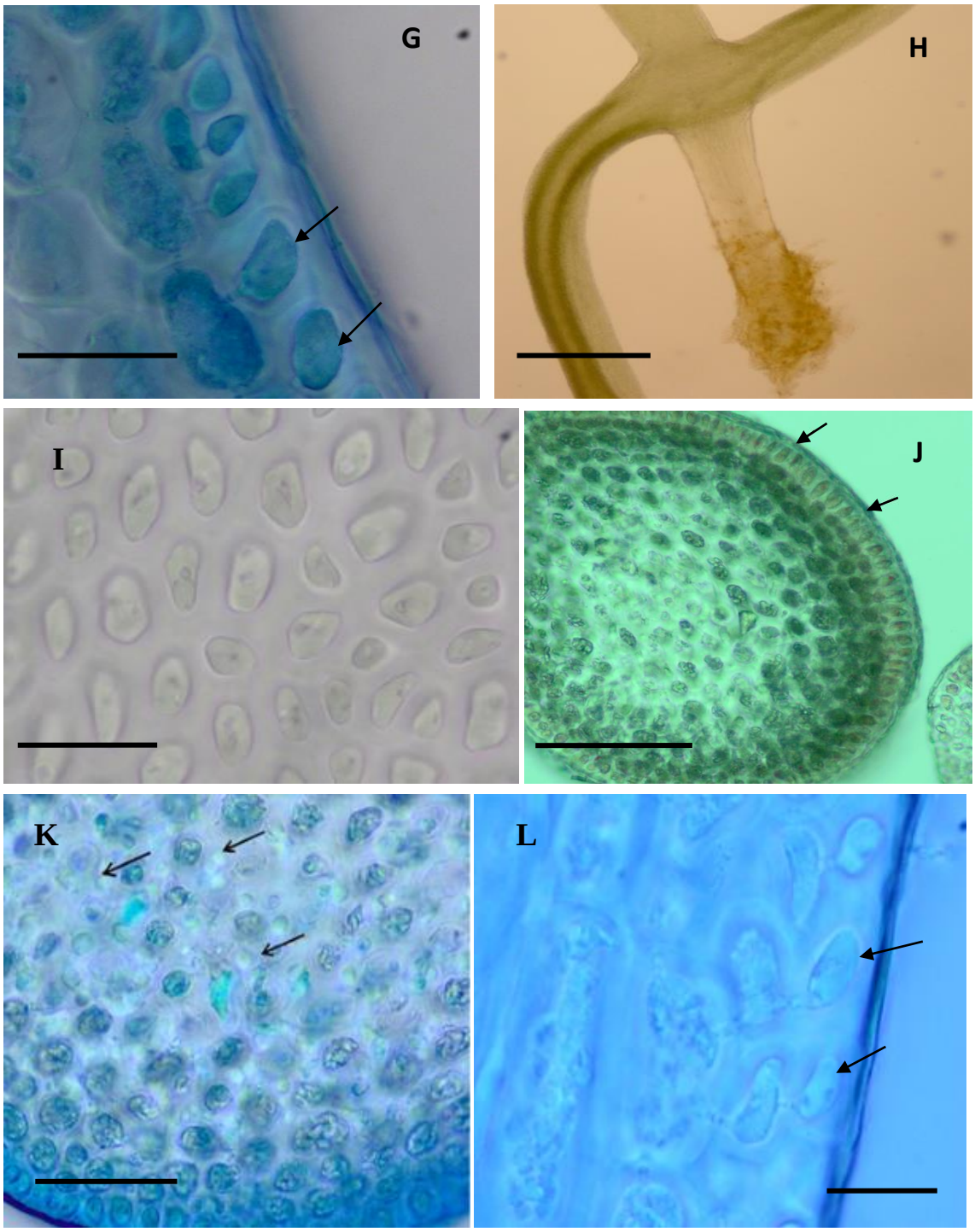

Figure 4.3: *Pterocladiella caerulescens* (Kützing) Santelices et Hommersand

(G) Oblique outermost cortical cells (arrows) and vertical inner cortical cells in longitudinal section of erect axis. Scale bar = 20 µm, (H) Peg-like rhizoid. Scale bar =500 µm, (I) Irregular and highly spaced cortical cells in surface view of stolon. Scale bar = 20µm, (J) Anticlinal outermost cortical cells (arrows), periclinal inner cells medullary cells in transverse section of stolon. Scale bar = 100 µm, (K) Scattered distribution of rhizines among medullary cells (arrows) in transverse section of stolon. Scale bar = 50µm. (L) Oblique outermost cortical cells (arrows) in longitudinal section of stolon. Scale bar = 20 µm.

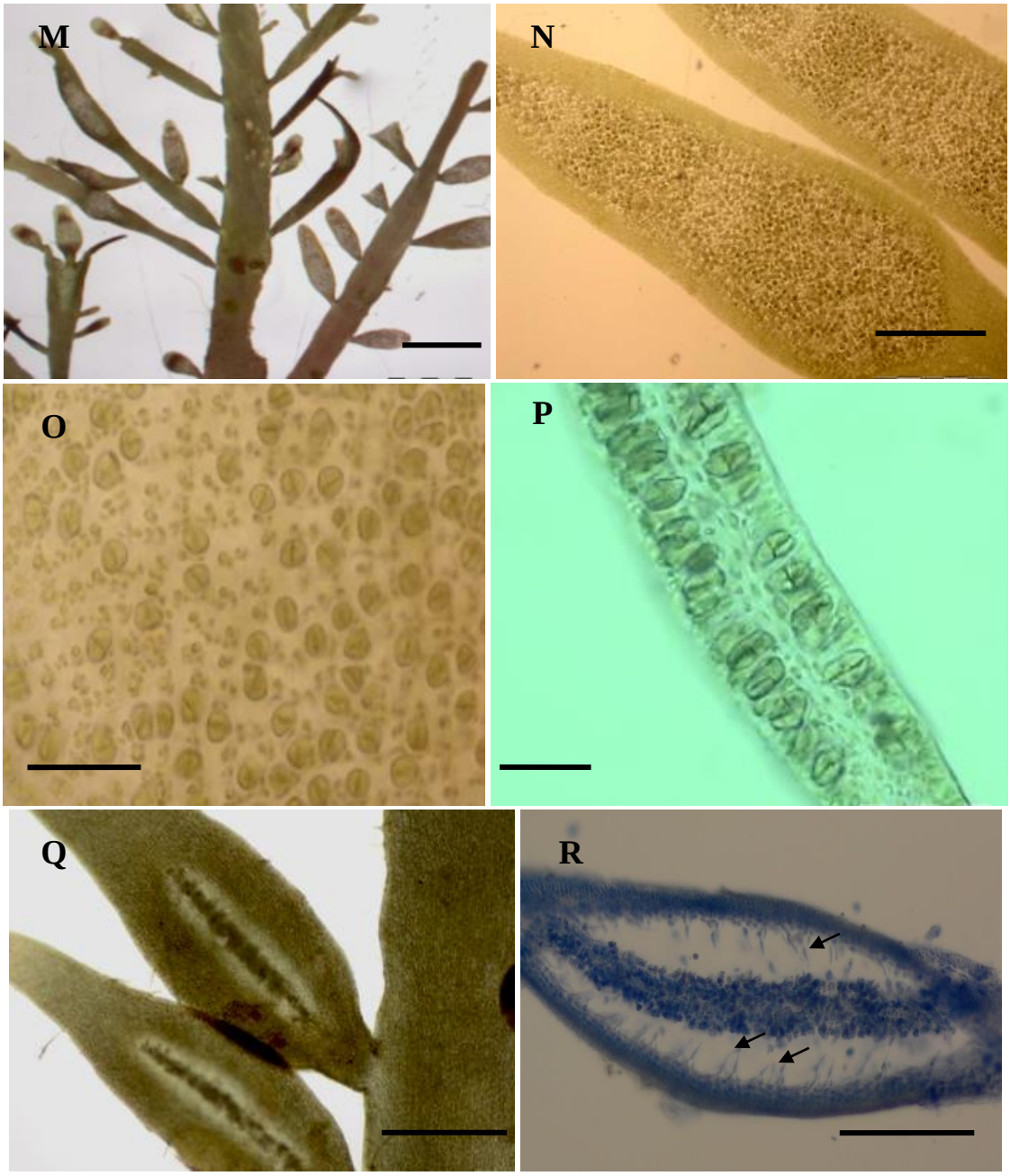

Figure 4.3: *Pterocladiella caerulescens* (Kützing) Santelices et Hommersand (continue).

(M) Ovoid tetrasporangial sori on lateral branches. Scale bar = 2 mm, (N) Irregular arrangement of tetrasporangia and sterile margin of tetrasporangial sorus in surface view. Scale bar = 500 µm, (O) Close up of irregular arrangement of tetrasporangia. Scale bar=100 µm, (P) Transverse section of tetrasporangial sorus. Scale bar = 100 µm, (Q) Cylindrical cystocarp on lateral branches with sterile margins. Scale bar = 500µm, (R) Longitudinal section of cystocarp shows a dense chain of central placenta is connected by nutritive filaments to cystocarp walls. Scale bar= 200µm.

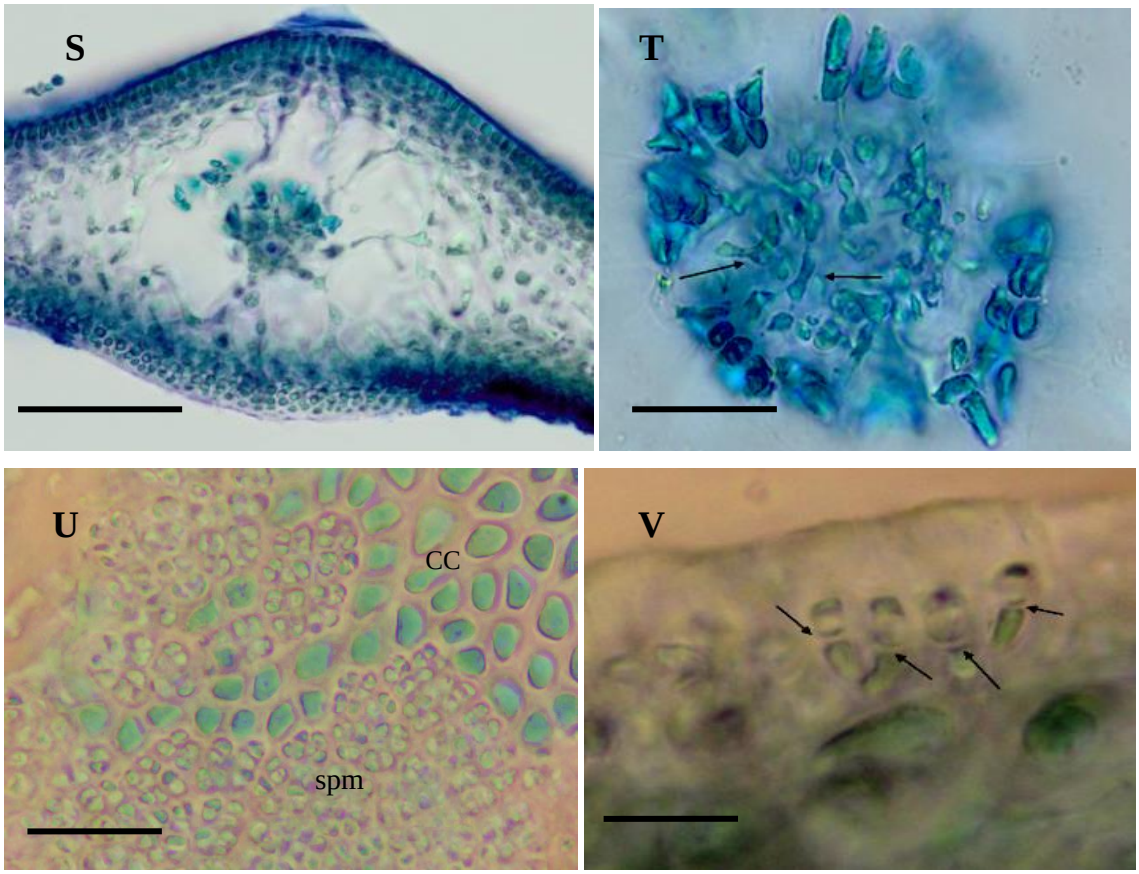

Figure 4.3: *Pterocladiella caerulescens* (Kützing) Santelices et Hommersand (continue).

(S) Transverse section of cystocarp shows second and third order of nutritive filament supporting gonimoblasts. Scale bar = 100 µm, (T) Central placenta and connection of nutritive filaments to precarp in transverse section of cystocarp. Scale bar = 10 µm, (U) Surface view of large spermatangial patches among cortical cells of cystocarp. Scale bar = 20 µm, (V) Transverse section through spermatangial sorus shows transverse division (arrows) of spermatangial initial cells (arrows). Scale bar = 10 µm.

4.1.1.4 *Pterocladiella* sp. nov. 1 (Figs 4.4)

Plants up to 1 cm high, purple to yellowish in colour. Erect axes arising from prostrate terete branches, attached to the substratum by rhizoidal peg-like haptera, cylindrical at base and semi-compressed in middle to upper parts. Branching irregular to alternate, up to three orders of branching; branching mostly at right angles to main axis. Branches linear-lanceolate to ligulate, tapering toward apices and ending in acute tips with dome-shaped apical cells; rarely constricted at the base except when bearing lateral ligulate tetrasporangial stichidia; width and length of older branches similar to main axes, resulting in a corymbose habit. Erect axes up to 240 µm in width with 2-3 layers of cortical cells, outermost layer with anticlinal arrangement; abundant translucent rhizines surrounding 1-3 transverse rows of small medullary cells. Stolons terete, 55-110 µm in diameter with peg-like rhizoids, 2-3 layers of periclinally arranged cortical cells, and rhizines surrounding the small medullary cells. Peg-like attachments sometimes observed on erect branches. Tetrasporangial sori subterminally placed on ultimate ligulate branches and branchlets and occasionally intercalary on main axis; tetrasporangia arranged in V-shape in young sori, becoming irregular in mature tetrasporangial sori with sterile margins. Reproductive gametophytes not observed.

Holotype: PSM12598-1 (Fig. 4), coll. J. Sohrabipoor, 29 Aug. 2011.

Paratype: PSM12598-2, coll. J. Sohrabipoor, 29 Aug. 2011.

Type locality: Port Dickson, Negeri Sembilan (2° 24' 54" N; 101° 51' 10" E), Malaysia.

Ecology: Plants are minute and epilithic, growing on rocks and sand-covered boulders exposed to waves in the mid-intertidal zone. The prostrate attachment system of the plant is tightly attached to the rock and is collected only by scraping.

Specimens examined: Holotype, Port Dickson, Negeri Sembilan, Malaysia; 29 Aug. 2011, J. Sohrabipoor, PSM12598-1, PSM12598-2; Port Dickson, Negeri Sembilan (2° 24' 54" N; 101° 51' 10" E), 30 Dec. 2009, J. Sohrabipoor, PSM12502-1, PSM12502-2, and PSM12502-3; Port Dickson, Negeri Sembilan, 30 Dec. 2009, J. Sohrabipoor, PSM12504, PSM12504-1, and PSM12504-2.

Description: Thalli up to 10 mm tall and purple to yellowish in colour, consisting of stolons and erect axes (Figs. 4.4A & 4.4B). Erect axes arising from stolons posterior to attachment point to the substratum, cylindrical at base, 50-110 µm in diameter and gradually increased upwardly and becoming semi-compressed, to 240 µm in width and up to 85 µm thick. Branching up to three orders, branching pattern irregular to alternately distichous, with new branches and tetrasporangial branchlets initiated at right angles to main axis (Figs. 4.4A & 4.4B). Basal and lower lateral branches growing as tall as main axis and resulting in a corymbose habit (Figs. 4.4A & 4.4B). Basal constrictions were uncommon and only observed in young branches and tetrasporangial stichidia (Fig. 4.4B). Older branches were semi-compressed, linear lanceolate, while young branches were ligulate to lanceolate (Figs. 4.4A & 4.4B) tapering to apices with dome-shaped apical cells, which after one to three transverse divisions followed by longitudinal division, produced two lateral and one axial cells (Figs. 4.4C & 4.4D). In surface view of erect axes, cortical cells were small, rounded to ovoid, 5.2-11.5 x 3.3-9.2 µm and arranged irregularly (Fig. 4.4E). In transverse sections of erect axes and branches, 2-3 layers of cortical cells were observed, outermost cells isodiametric to

ovoid, anticlinally arranged, 5.4-8.8 x 4.5-6.6 µm and gradually becoming larger inwardly, 5.1-10.7 x 5-7 µm and periclinally arranged (Fig. 4.4F). Medulla consisting of 1-3 transverse rows of small rounded medullary cells and abundant translucent rhizines, 3.6-6.2 µm in diameter (Fig. 4.4F). In longitudinal section, erect axes and branches consisted of 2-3 layers of cortical cells (Fig. 4.4G), the outermost oval-shaped cortical cells being arranged obliquely, 3-9 x 3-6.7 µm, the inner cells arranged vertically, 9-13 x 5-7 µm and medulla consisting of long colourless cells and rhizines.

Stolon were cylindrical, 55-110 µm in diameter, attached to the substratum by coalescent rhizoidal peg-like haptera 50-100 µm in diameter and 260-708 µm in height, surrounded by multicellular filaments originating from outer cortical cells (Fig. 4.4H). Cortical cells of stolons in surface view were rectangular to polygonal and larger than the cortical cells of erect branches, 6.3-18 x 4-10.7 µm and longitudinally to irregularly arranged (Fig. 4.4I). In transverse section stolons had 2-3 layers of cortical cells and the medulla showed lower number of rhizines compared to erect axes; cortical cells were elliptical to rounded, occasionally isodiametric and all layers were periclinally arranged (Fig. 4.4J); outermost cortical cells were 5.9-10.8 x 3.4-7.3 µm; inner cortical cells became larger, 8-16 x 5.3-10 µm, innermost cells were 12-16 x 7.5-10 µm and low number of small medullary cells, 5.2-8.8 in diameter, were surrounded by small rhizines (Fig. 4.4J). In longitudinal section, stolon consisted of 2-3 vertical layers of cortical cells (Fig. 4.4K); the outermost cells were rounded to rectangular, 5-10.2 x 4-8.5µm and the inner cells 11.4-31.7 x 5-11.5 µm while the medulla consisted of long colourless cells and rhizines.

Tetrasporangial sori developed on the sub-apical to mid part of lateral stichidia and branches and sometimes on the main axes, 365-1290 x 100-380 µm and 50-118 µm

thick with entire sterile margin (Figs. 4.4L). Tetrasporangia disposed in V-shaped arrangement in young sori and became slightly irregular in mature and older sori (Fig. 4.4L), 12-26 µm diameter in surface view and 22-41 x 14-30 µm in transverse section (Fig. 4.4N). A sterile margin was a distinctive feature on both sides of tetrasporangial sorus, up to 50 µm in width. In transverse section of tetrasporangial stichidia, rosette complexes of rhizines were observed in the margins at the basal part of the sorus (Fig. 4.4O). Gametophytic plants were not observed.

Diagnostic features: Minute size; lateral branches originating at right angles to main axis, obscure main axis in mature thalli, corymbose habit of plant, sterile entire margin of the tetrasporangial sori, periclinal arrangement of all cortical layers of stolons, 1-3 transverse rows of small medullary cells and abundant rhizines in medulla of erect axis and stolon were the distinctive characteristics of *Pterocladiella* sp. nov.1.

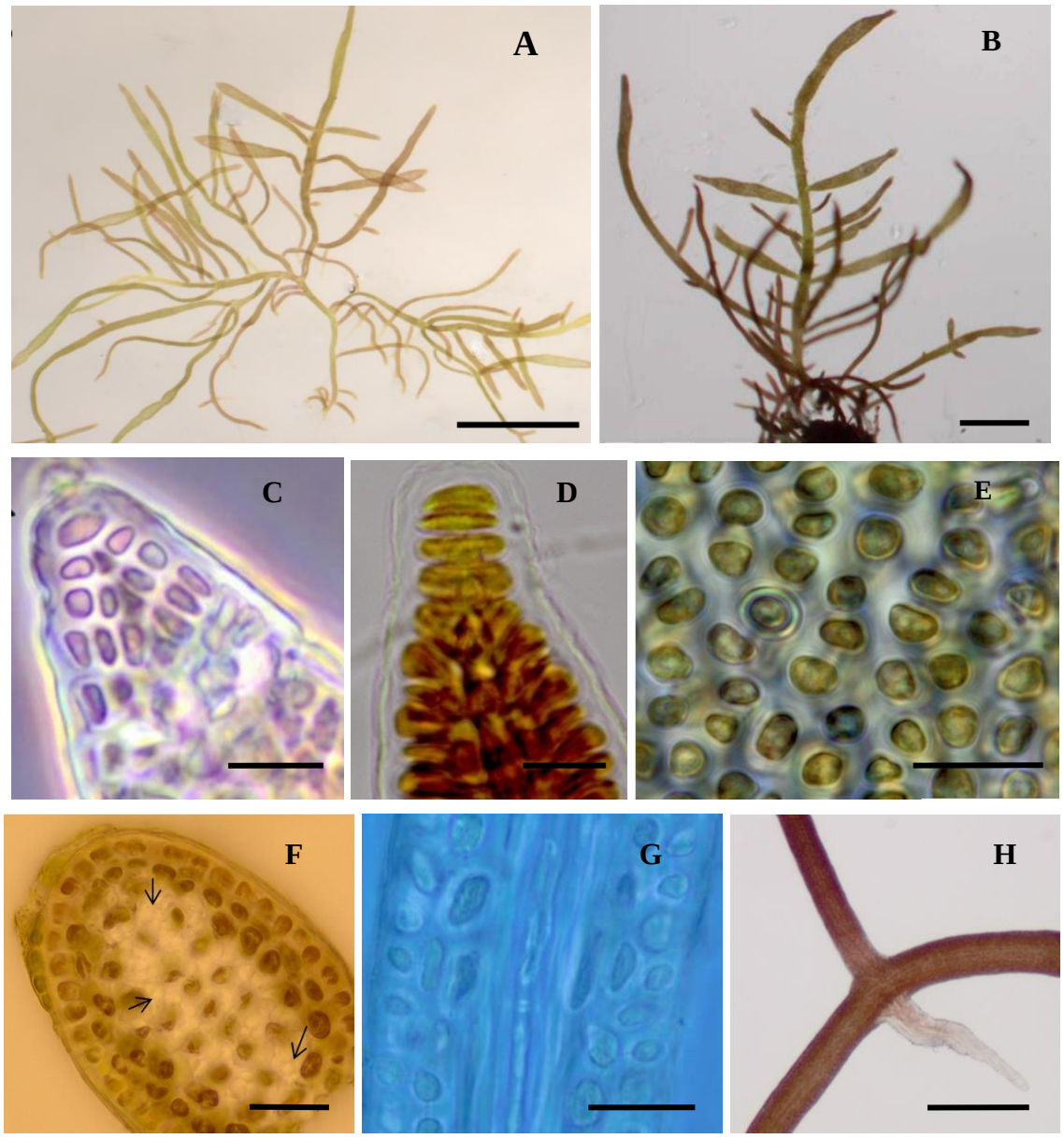

Figure 4.4: *Pterocladiella* sp. nov.1

(A) Holotype (PSM12598-1). Habit of thallus with irregular branching and long lateral branches. Scale bar = 2 mm, **(B)** Right-angled branching in alternate to opposite distichous pattern and constriction at base of young branches. Scale bar = 1 mm, **(C)** Dome-shaped apical cell at apices of axes and branches. Scale bar = 10 µm, **(D)** Successive transverse divisions of apical cell. Scale bar = 10µm, **(E)** Surface view of irregular arrangement of rounded to ovoid cortical cells in erect axes. Scale bar = 20 µm, **(F)** Anticlinal arrangement of outermost cortical cells and periclinal arrangement of inner cells with abundant translucent rhizines surrounding 1-3 transverse rows of medullary cells in cross section of erect axis. Scale bar = 50 µm, **(G)** Oblique arrangement of outermost cortical cells and vertical direction of inner cortical cells and medullary cells in longitudinal section of erect branches. Scale bar = 20 µm, **(H)** Cylindrical stolon and peg-like rhizoid. Scale bar = 200 µm.

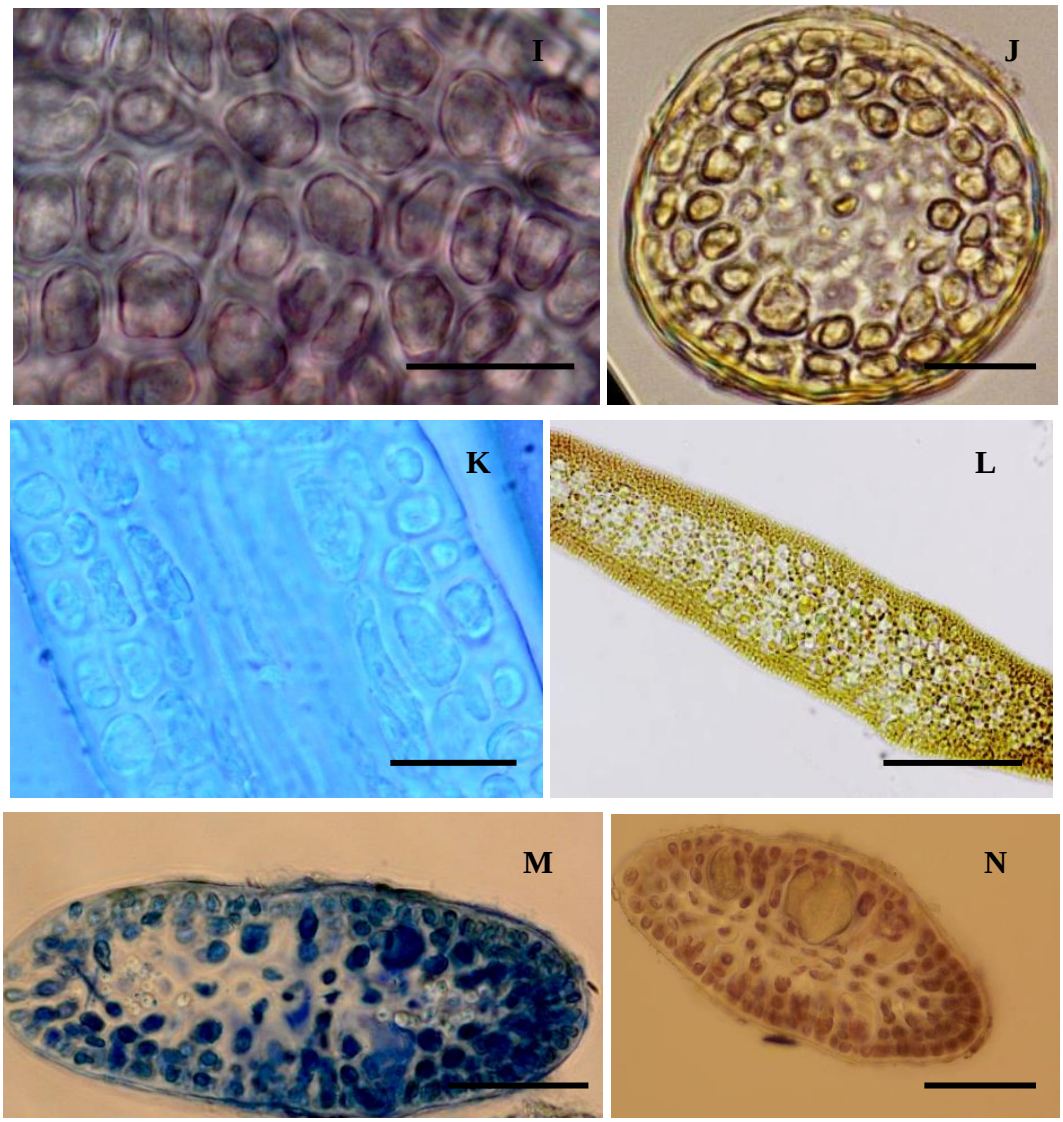

Figure 4.4: *Pterocladiella* sp. nov.1

(I) Rectangular to polygonal cortical cells with longitudinal (parallel to stolon axes) to irregular arrangement in surface view of stolon. Scale bar = 20 µm, **(J)** 2-3 layers of periclinally arranged elliptical to rounded cortical cells in transverse section of stolon. Scale bar = 50 µm, **(K)** 2-3 layers of vertically arranged rectangular to elongate cortical cells in longitudinal section of stolon. Scale bar = 20 µm, **(L)** Irregular arrangement of tetrasporangia in mature tetrasporangial sorus with sterile margin. Scale bar = 200 µm, **(M)** V-shaped arrangement of tetrasporangia in young tetrasporangial sorus. Scale bar = 500 µm, **(N)** Transverse section of tetrasporangial sorus with 3 rows of transverse medullary cells. Scale bar = 50 µm, **(O)** Transverse section from basal part of tetrasporangial stichidia shows rosette complex of rhizines in sterile margins. Scale bar = 50 µm.

4.1.1.5 *Pterocladiella* sp. nov. 2 (Fig. 4.5)

Plants up to 5mm tall, purple to olive green in color, composed of erect axes arising from a semi-compressed to compressed stolon. Erect axes cylindrical at base, semi-compressed to compressed in mid-section, 76-314 µm in width and tapering distally. Branching rare, irregular, mostly unilateral and sometimes polytrichous on the blunt and injured apex of main axes; one order of branching. Branches linear to lanceolate, mostly with terminal lanceolate tetrasporangial sorus. Erect axis composed of two to three cortical cell layers and one row of transverse small medullary cells with few rhizines near medullary cells.

Stolons composed of two periclinal layers of cortical cells and three rows of large medullary cells and rare small rhizines among medullary cells; attached to substratum by peg-like rhizoidal holdfast with discoid end.

Tetrasporangial sori on terminal part of long or short lateral branches and apex of main axes or on apex of regenerated branchlets; sorus sometimes produced by whole unbranched erect axis; tetrasporangia disposed in V-shaped rows in young and mature sori with no sterile margin. Gametophytic reproduction not observed.

Holotype: PSM12599-1 (Fig. 4.1.19), coll. J. Sohrabipoor, 29 Aug. 2011.

Type locality: Teluk Kemang, Port Dickson, 2 ° 26' 38" N; 101 ° 51' 21" E), Malaysia.

Ecology: Plants are epilithic and grow on small pieces of coral among *P. beachiae* and *P. caerulescens* population in the mid-intertidal zone of Teluk Kemang, Port Dickson, Malaysia.

Specimens examined: Holotype, Teluk Kemang, Port Dickson, Negeri Sembilan (2 ° 26' 38" N; 101 ° 51' 21" E), Malaysia, 29 Aug. 2011; J. Sohrabipoor, PSM12599-1; Teluk Kemang, Port Dickson, Negeri Sembilan (2 ° 26' 38" N; 101 ° 51' 21" E), 29 Aug. 2011, PSM12600-1, PSM12601-1. PSM12602, PSM12603; Pulau Pinang (N 5 ° 27' 54" N; 100 ° 13' 23" E), 5 Feb. 2012, PSM12628.

Description: Plants are up to 5 mm tall, purple to olive green in color; composed of stolons and erect axes (Figs. 4.5A-C). One to three erect axes arise from the same point of the stolon opposite the peg-like rhizoidal holdfast. Base of erect axes cylindrical to semi-compressed and compressed, slightly flattened in upper parts (Fig. 4.5C), 76-314 µm broad and 43-60 µm thick. Branching is rare, irregular and sometimes unilateral in upper parts of axes and polytrichous at blunt or injured apex, one order of branching (Figs. 4.5A-C). Basal constriction not observed. Erect axes lanceolate, tapering distally to acute to rounded tips (Fig. 4.5A-C) with dome-shaped apical cell (Fig. 4.5D) dividing transversely followed by longitudinal division to produce two laterals and one axial cell.

Stolons semi-compressed (Fig. 4.5E), 53-132 µm in width, 64-88 µm thick and producing peg-like holdfasts consisting of parallel coalescent rhizoidal filaments surrounded by cortical filaments, 43-110 µm in diameter and 180-464 µm in length, with discoid haptera at the attachment point, consisting of coalescent parallel rhizoids (Figs. 4.5E & 4.5F).

Cortical cells in surface view of erect branches are polygonal to ovoid, 4.8-14 x 3.2-8.2 µm, and irregularly arranged (Fig. 4.5G). In transverse section, erect axis and branches mostly composed of two or sometimes three layers of cortical cells and one row of medullary cells. Outer cortical cells ovoid to rounded, 4.2-7.6 x 3.4-6 µm, and

arranged anticlinally. Inner cortex cells larger and mostly isodiametric, 5.8-8.4 x 5.1-7.4 µm. Medulla consisting of rounded cells arranged in one transverse row (Fig. 4.5H). Rhizines small, 3.9-4.6 µm, few, near medullary cells.

In longitudinal section erect axes showed two layers of cortical cells with elliptical to ovoid outer cells, 4.7-11 x 3-5.7 µm, obliquely to vertically arranged and inner cells rounded to elliptical, 7.3-14.8 x 5-8.1 µm and arranged vertically; medulla containing one layer of long vertically arranged medullary cells 17.2-41.2 x 3.6-5.1 µm, with distinct pit plug between upper and lower cells (Fig. 4.5I).

Cortical cells in surface view of stolon polygonal, 5.7-12 x 4.6-8.9 µm (Fig. 4.5J), and in transverse section stolon consisted of two layers of cortical cells; medulla composed of large medullary cells in three transverse rows (Fig. 4.5K), both cortical cell layers arranged periclinally. Outermost cortical cells elliptical to rounded, 4.8-9.5 x 3.5-7.8 µm, and inner cortex cells were larger, 8.5-11.5 x 6.1-7.9 µm. Three rows of large medullary cells, 7.7-12.1 x 5.3-10.9 µm, arranged transversely (Fig. 4.5K). A small number of rhizines, 3-4.3 µm in diameter, observed among medullary cells.

Two layers of cortex cells observed in longitudinal section of stolon (Fig. 4.5L); outermost cells were elliptical to oval and sometimes triangular, 4.5-6.5 x 3.5-4.5µm and were arranged vertically to obliquely. Inner cells larger, 5.9-11.8 x 4.6-7.9 µm, rounded to elliptical or irregular in shape, arranged vertically, and medulla contain 3-4 layer of long vertically arranged medullary cells 23.8-44.6 x 4.2-5.9 µm. Pit plugs enlarged between medullary cells (Fig. 4.5L).

Tetrasporangial sori lanceolate to long-lanceolate in shape, mostly developed acropetaly on distal parts of lateral and apical branchlets and sometimes forming over whole length of erect axes (Figs. 4.5A-C & 4.5M-N), 165-1300 µm in length and 115-235 µm in width and up to 98 µm in thick, without sterile margins (Fig. 4.5N); tetrasporangial arrangement in young and mature sori and after spore shedding was V-shaped. Tetrasporangia large, 26.6-37µm diameter in surface view and 26-42 x 21.6-37 µm in transverse section. Gametophytic plants were not observed.

Diagnostic features: Very minute size, semi-compressed to compressed stolons and erect axes, unilateral and sometimes polytrichous apical branches, one order of branching, two layers of cortical cells in stolons and erect axes with few rhizines among medullary cells; single row of medullary cells in erect axis, periclinal arrangement of all cortical cell layers in stolons, rhizines rare among large medullary cells of stolons, absence of sterile margin and large tetrasporangia are the distinctive characteristics of *Pterocladiella* sp. nov. 2.

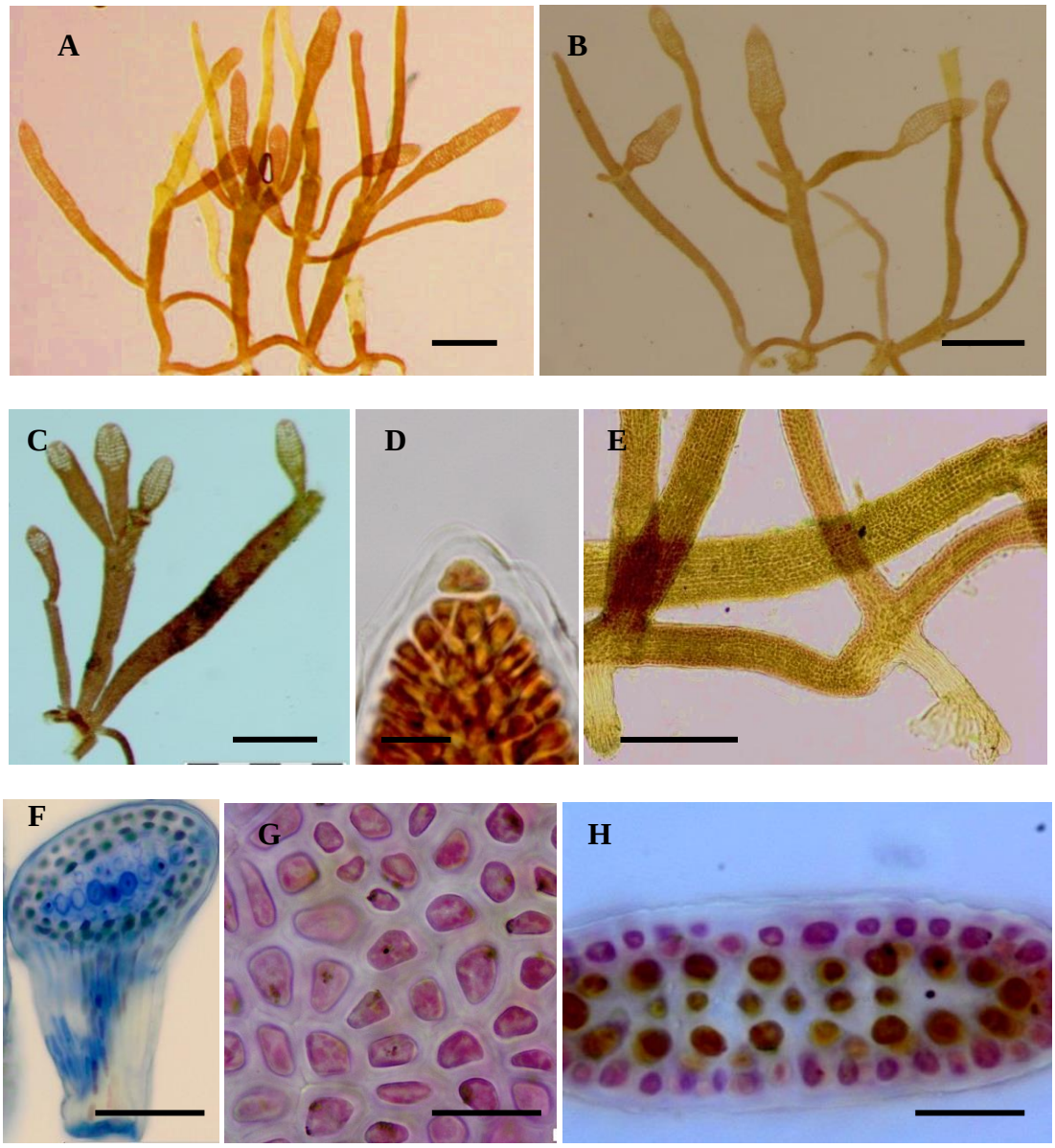

Figure 4.5: *Pterocladiella* sp. nov. 2

(A) Habit of holotype (PSM12599-1). Lateral and apical polytrichous branching with terminal tetrasporangial sori. Scale bar = 1 mm, **(B)** Rare branching and terminal tetrasporangial sori on apical part of axes and absence of constriction in basal parts of branches and branchlets. Scale bar = 1 mm, **(C)** Habit of thallus showing rare branching and absence of constriction. Scale bar = 500 µm, **(D)** Dome-shaped apical cell. Scale bar = 20 µm, **(E)** Semi-compressed stolon and rhizoid with discoid haptera. Scale bar = 200 µm, **(F)** Longitudinal section of peg-like rhizoid shows coalescent rhizoidal filaments. Scale bar = 100 µm, **(G)** Irregular arrangement of polygonal cortical cells in surface view of erect axes. Scale bar = 20 µm, **(H)** Two layers of cortical cells and one transverse row of medullary cells in transverse section of erect axes. Scale bar = 20 µm.

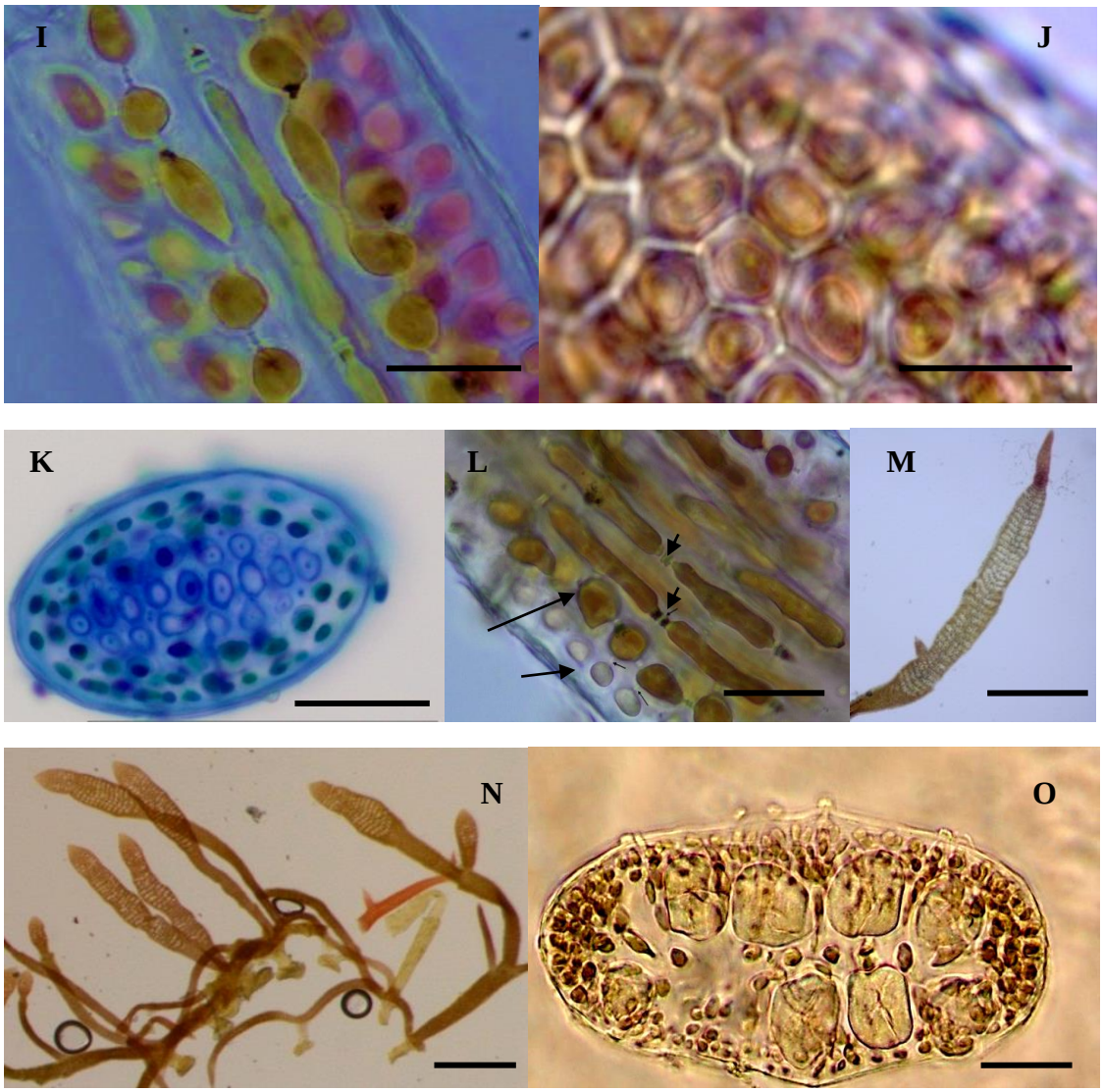

Figure 4.5: *Pterocladiella* sp. nov. 2

(I) Two layers of cortical cells with oblique elliptical outer cortical cells and vertically arranged inner cortical cells. Scale bar = 20 µm, (J) Polygonal cortical cells in surface view of stolon. Scale bar = 20 µm, (K) Two layers of periclinally arranged cortical cells and 3 transverse rows of large medullary cells in transverse section of stolon. Scale bar = 40 µm, (L) Longitudinal section of stolon showing two layers of cortical cells (arrows) and three layers of vertical medullary cells with obvious pit plugs (arrow heads). Scale bar = 20 µm, (M) Long lanceolate sorus with V-shaped tetrasporangia. Scale bar = 1 mm, (N) V-shaped tetrasporangial sorus after spore shedding. Scale bar = 500 µm, (O) Large tetrasporangia in transverse section of tetrasporangial sorus. Scale bar = 40 µm.

4.1.1.6 *Aphanta* sp. (Figures 4.6)

Plants up to 1cm long, reddish to pink in colour; arising from robust, stoloniferous holdfast; upright axes cylindrical at base and abruptly became flattened, up to 1.3mm wide, and up to 87 µm thick. Branching rare, opposite to irregular, up to two orders; branches ovate, ligulate to lanceolate, basally constricted, with mucronate apices when young, to rounded and obtuse in well developed thalli. Stolon terete, up to 250 µm in diameter and usually extensively branched, attached to substratum by unvorticated peg-like and brush-like rhizoids, peg-like rhizoids also were branched and formed a fibrous attachment system. In transverse sections erect axis composed of 2-3 layers of isodiametric cortical cells, outermost cortical cells 5.9-8.0 x 4.9-6.3 µm, inner cortical cells 9.4-14.0 µm and thick-walled medullary cells were 8.0-12.0 µm in diameter; rhizines 3.0-5.5 µm in diameter, and scattered among medullary cells; cell dimensions are greater in the stolon. Reproductive plants were not observed.

Small size, extensively branched system of stolon and peg-like rhizoids and also rare branching of erect axis and mucronate tips are the distinctive features of the species compared with *Aphanta pachyrrhiza* Tronchin & Freshwater, the sole reported species of the genus *Aphanta* from South Africa.

Ecology: Plants of the species grow in the crevices of the rocks or on artificial stable substrate like plastic ropes forming small reddish patches.

Specimens examined: Teluk Kemang, Port Dickson, Negeri Sembilan, 2°26 ' 38 " N/ 101° 51 ' 21 " E) Malaysia, 29 July 2020; Reza Rabiei, PSM12539-1; Teluk Kemang, Port Dickson, Negeri Simbilan, 2°26 ' 38 " N/ 101° 51 ' 21 " E) Malaysia, 29 July 2020; Reza Rabiei, PSM12539-1-4 (slides); Teluk Kemang, Port Dickson, Negeri Simbilan,

2˚ 26 ' 38 " N/ 101˚ 51 ' 21 " E), 29 Augst 2011, Reza Rabiei & J. Sohrabipoor, PSM12586, PSM12587; Pulau Manukan (5˚ 32' 27.68 " N, 116 ˚ 00 '11.34 " E) Kota Kinabalu, Sabah, 3 Aug. 2012, J. Sohrabipoor & R. Rabiei, PSM12679.

Description: Plants of the species were collected from the small patches growing in crevices of the rocks in middle intertidal zones and from the thick plastic ropes found at the small local jetty in Pulau Manukan, Kota Kinabalu, Sabah. Plants up to 1cm tall, reddish to pink in colour; consisting of the extensively branched robust stolon and rhizoids which penetrate into the crevices of the rocks. Flattened erect branches with a short slender base which abruptly flatten out and give rise to the long ovoid to ligulate erect axes (Figs. 4.6A, 4.6B & 4.6D), up to 1.3mm wide and 87 µm thick. Branching rare, opposite to irregular up to two orders (Figs. 4.6.A-D), and injured thalli show more irregular branching in erect axes (Fig. 4.6C).

Stoloniferous prostrate branches showed a high degree of irregular branching in uncorticated rhizoids (Figs. 4.6B, 4.6C & 4.6E) in comparison with other species of the family Pterocladiaceae and even when compared to *Aphanta pachyrrhiza.* Erect axis and branches ovate, ligulate to lanceolate (Figs. 4.6A, 4.6B & 4.6D), basally constricted, ending in rounded to mucronate apices with dome-shaped apical cell (Figs. 4.6F & 4.6G), lateral apical cells in margins of the apical part were also observed (Fig. 4.6G). Thin hyaline hairs were common on the terminal parts of the young erect axes among the marginal apical cells (Fig. 4.6H). In surface view of erect branches, small quadrate to angular cells, 5.5-8.8 x 3.1-5.6 µm, were arranged irregularly (Fig. 4.6I). Cells dimensions in surface view of stolons were greater than the erect axes, arranged transversely and contain pale discoid granulated pigments (Fig. 4.6J). In transverse section of erect axis, cortex composed of 2-3 layers of cortical cells with outermost

cortical cells, 5.9-8.0 x 4.9-6.3 µm, isodiametrically arranged, inner cortical cells 9.4-14.0 µm and thick-walled colorless medullary cells were 8.0-12.0 µm in diameter (Fig. 4.6K). Low number of small translucent rhizines, 3.0-5.5 µm in diameter, scattered among medullary cells (Fig. 4.6K). Longitudinal section of erect branches showed 2- 3 layers of cortical cells, outermost cortical cells arranged isodiametrically or longitudinal and inner cells obliquely flanked medullary colorless cells (Fig. 4.6L).

Stolon terete, robust, up to 250 µm in diameter and usually extensively branched to being entangled; peg-like holdfasts also branched and form a fibrous attachment system (Figs. 4.6B, 4.6C & 4.6E). No evidence of brush-like attachment and mostly were peg-like haptera. Reproductive plant was not observed.

Diagnostic features: Small size, extensively branched system of stolon and peg-like rhizoids and also rare branching of erect axis and mucronate tips are the distinctive features of the species compared with *Aphanta pachyrrhiza* Tronchin & Freshwater, the sole reported species of the genus *Aphanta* from South Africa.

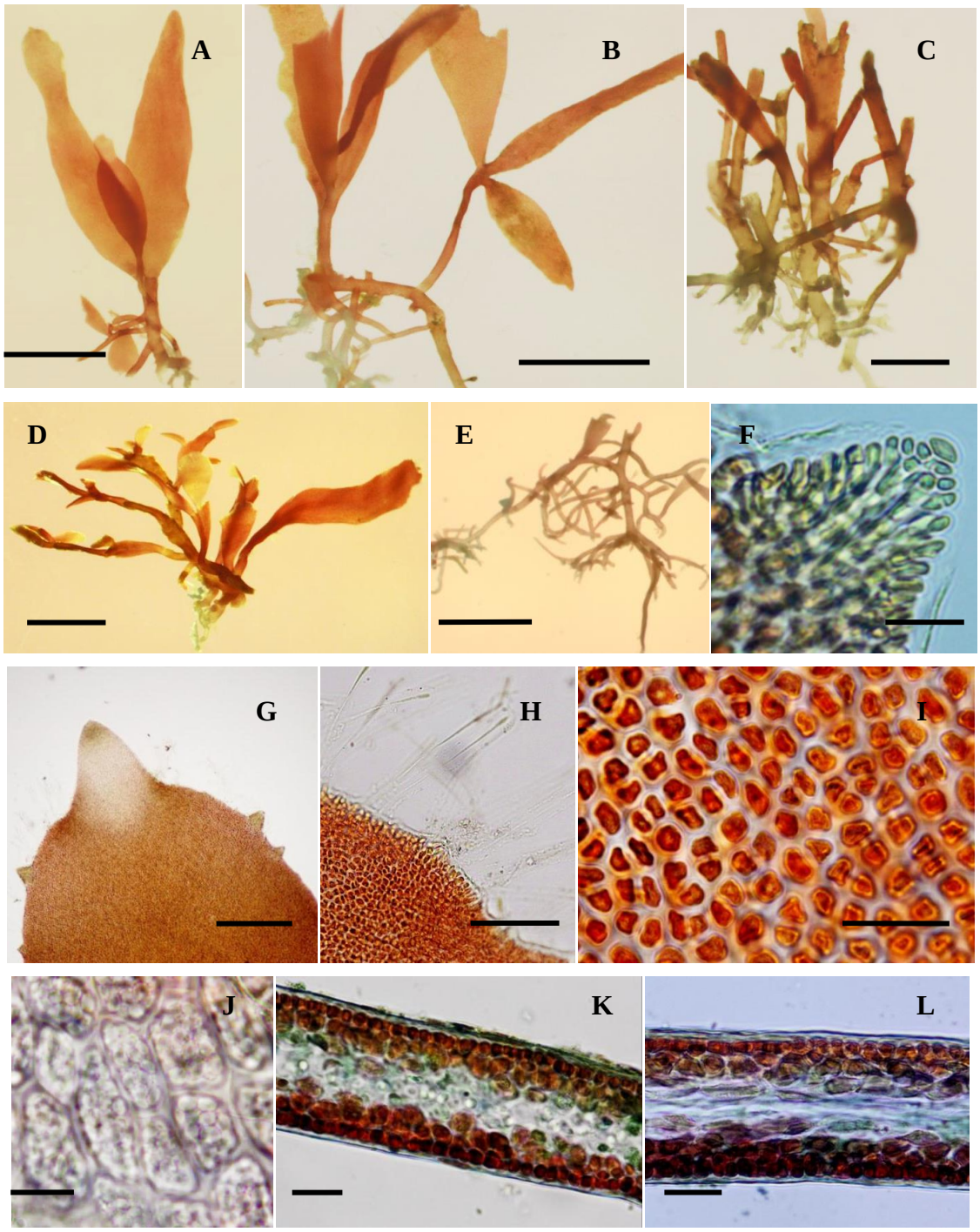

Figure 4.6: *Aphanta* sp.

(A) Habit of plant, rare branching in erect axes. Scale bar = 2 mm, (B) Habit of plant, rare distichous to irregularly branched axis. Scale bar= 2 mm, (C) Abundant and irregular branching in erect axes, stolon and rhizoids. Scale bar = 2mm, (D) Ligulate and ovate form of axes and branches. Scale bar = 2mm, (E) Abundant branching in stolon and Peg-like rhizoids. Scale bar =2 mm, (F) Mucrunate apices and lateral apical cell. Scale bar = 500 µm, (G) Dome-shaped apical cell. Scale bar=50 µm, (H) Marginal hyalin hair on erect axis. Scale bar = 50 µm, (I) Angular and quadrate cortical cells in surface view of erect axes. Scale bar = 20µm, (J) Transverse arrangement of cortical cells in surface view of stolon. Scale bar = 20µm, (K) Isodiametric outermost cortical cells and low number of rhizines in transverse section of erect axis. Scale bar = 20 µm, (L) Longitudinal section of erect axes. Scale bar = 20µm.

Table 4.1: Morphological comparison of *Pterocladiella* species

Species / **Character**	***Pterocladiella* sp. nov.1**	***Pterocladiella* sp. nov.2**	***P. minima*[1]**	***P. caespitosa*[2]**	***P. taylorii*[3 & 4]**
Height	up to 1cm	up to 0.5cm	1-1.5 cm	up to 1cm	2-3 cm
Habit	corymbs	Uniaxial to obpyramidal	ni	ni	ni
color	purple to yellowish	purple to olive green	red-brown	ni	Pale red or pink
Stolon form	Cylindrical	semicompressed to compressed	Cylindrical to ompressed	cylindrical	cylindrical to semicompressed
stolon width & thick (µm)	55-110	54-132/ 64-88	50 -200 / 40-100	in	100-150/ ni
Branches form	Long lanceolate to ligulate	Long linear to lanceolate	ni	ni	ni
Erect axis (base /upper)	Cylindrically /semicompressed	semicompressed / compressed,	terete to compressed	slightly flattened,	compressed
Erect axis(width/ thick (µm)	55-240 / 85	76-314 / 43-60	50- 200/40-100	Up to 1000/ ni	310 -550/ ni
Branching	Irregular to alternately distichous	irregular, unilateral to polytrichous	unbranched	Unbranched or rare at apices	rare
order of branching	up to 3 order	one order	unbranched	one order	up to 2 order
Tetrasporangial sorus	long ovoid	Lanceolate to long lanceolate,	terminal, paired	at the expanded tips	scattered
Tetrasporangia sori (LxW)(thick)(µm)	Up to 1290 x 340/ 118	Up to1300 x 235/ 98	Up to1000 x 300	ni	ni
Tetrasporangia (diam./Lx W) (µm)	12-27 / 22-41 x 14-31.7	26.6-37 /26-42 x 21.6-37	23-45/ni	ni	ni
Tetrasporangia arrangement	V-shape to irregular	V-shaped	in regular rows	V-shaped to irregular	V-shaped
Tetrasporangial margins	Present	Absent	Absent	Absent	Absent
Axis cortical cells in surface view	Rounded to ovoid , 5-11 x 3-9 µm	Polygonal to ovoid, 5-14 x 3-8 µm	rounded	ni	ni
Axis cortical cells layers	2-3	2	2-3	3	2
Axis outermost cortical cells	Isodiametric to anticlinal, 5-9 x 4-7µm	Anticlinal, 4-8 x 3-6 µm	Isodiametric, 5-10 µm	ni	Isodiametric, 6-8 µm
Axis Inner cortical cells	5-11 x 5-7µm, periclinal	8-11 x 6 -8 µm , periclinal	2.5 - 6	ni	ni
Axis medullary cells	smaller than inner cells in1-3 rows	smaller than inner cells/ one row	one row	Large in 4-5 rows	7-8 rows
Stolon cortical cells in surface	6-18 x 4-11 µm, polygonal	6-12 x 5-9 µm, polygonal	ni	ni	ni
Stolon cortical cells layers	2-3	2	2-3	3	ni
Stolon outermost cortical cells	6-11 x 3-7 µm, periclinal	5-9 x 3-8 µm, preclinal	ni	ni	6-8, isodiametric
Stolon inner cortical cells	8-16 x 7-10 µm, periclinal	8-11 x 6-8 µm, periclinal	ni	ni	ni
Stolon medullary cells	smaller than cortical cells	Larger than cortical cells, 3rows	ni	Large in 4-5 rows	ni
Rhizines distribution	Abundant among medullary cells	Few among medullary cells	absent	scattered in medulla	scattered in medulla
rhizoid (height/diameter)	Peg-like, 260-708/50-100µm	Peg-like, 180–460/43-110	Peg-like, 80-160/40-80	Peg-like,	Peg-like, 100-200µm diam.
Cystocarp (L x w / thick)	ni	ni	Unilocular, 300 x 170 /200µm	Unilocular	ni
Type locality	Port Dickson, Malaysia	Teluk Kemang, Malaysia	Victoria, Australia	South Africa	Sao Paulo, Brazile

Sources of information: 1- Guiry & Womersley (1992); 2-Santelices (1998); 3-Taylor (1960); 4-Santelices (2007); 5- Feldmann & Hamel (1934), 6- Feldmann & Hamel (1936); 7-Guiry & Guiry (2012); 8-Taylor (1943); 9-Thomas & Freshwater (2001); 10-Santelices (1976); 11-Santelices (1977), 12- Santelices (1976); 13-Tronchin & Freshwater (2007);14-Barbára & Tapia (2012) (ni: no information).

Table 4.1 : Morphological comparison of *Pterocladiella* species (Continue)

Species / Character	***P. sanctarum*** [4 & 5]	***P. melanoidea*** [6, 7 & 14]	***P. bartlettii*** [2 & 8]	***P. bartlettii* (Malaysia)**
Height	2cm	2-3cm	6-8 cm	Up to 3 cm
Habit	ni	Corymbus to subfastigiat	Bushy to entangled	Bushy & entangled
color	purple	brownish- black	Dull purplish	Blakhish to pink
Stolon form	Cylindrical to semicompressed	Cylindrical	Cylindrical to compressed	Semicompressed
stolon width & thick (µm)	100- 120	ni	ni	84-216 / 44-116
Branches form	Tubular	Oblanceolate to linear lanceolate	Linear lanceolat to oblanceolate	Linear lanceolate to lanceolate
Erect axis (base /upper)	Cylindrical/ Semicompressed	Cylindrical/ compressed	Compressed to flattened	Cylindrical/ compressed/ flattened
Erect axis(width/ thick (µm)	up to 300/ 40-50	140-300 (-600)/150-200	Up to 600/ 125-170	Up to 603/up to 155
Branching	mostly unbranched	Divaricate/opposite to bipinnate	bilateral to polytrichous	bilateral to polytrichous
order of branching	1-2	Up to 3	1-2	2 - 3
Tetrasporangial sorus	lanceolate	Oblong to cylindrical	Enlarged Stichidiae	terminal or intercalary, expanded
Tetrasporangia sori (LxW)(thick)(µm)	450- 600 x 130-150	Up to 2000	ni	223–1920 x 86-320/ 127
Tetrasporangia (diam./Lx W) (µm)	ni/30-40 µm in length	ni/18-35 x 31-37	ni	10-37 x 6-25
Tetrasporangia arrangement	Irregular	V-shaped	V-Shaped/obscure	V-Shaped to scattered
Tetrasporangial margins	Absence	Presence	ni	Absence
Axis cortical cells in surface view	ni	8-20 x 6-15	ni	Rounded to conical,7-17x 4-9 µm
Axis cortical cells layers	2	3-4	3	2-4
Axis outermost cortical cells	Up to 12,isodiametric	5-8 x 6-12 µm , Isodiametric	ni	4-10 x 4-9 µm , isodiametric to periclinal
Axis Inner cortical cells	smaller than outer cells/ isodiametric	Larger than outer, Isodiametric	ni	9-18 x 10-16 µm, isodiametric/
Axis medullary cells	Smaller than inner cells/2-3 rows	Smaller than inner, /one row	ni	Smaller than inner, up to 3 row
Stolon cortical cells in surface	ni	ni	ni	Polygonal, 7-20 x 6-14 µm
Stolon cortical cells layers	ni	3-4	ni	3-4
Stolon outermost cortical cells	ni	isodiametric	ni	8-13 x 6-8 µm, periclinal
Stolon inner cortical cells	ni	Larger than outer, isodiametric	ni	13-25 x11-19µm, isodiametric to periclinal
Stolon medullary cells	ni	Smaller than inner	ni	Smaller than inner cells, up to 3 row
Rhizines distribution	Patchy, rare	Rare	Abundant in medulla	Scattered in medulla & rare in stolon
rhizoid (height/diameter)	Peg-like/200 µm / 100 µm	ni	Peg-like	Peg-like, 100-200µm in diameter
Cystocarp (L x w / thick)	ni	ni	Unilocular	ni
Type locality	Guadeloupe, West Indies	Morocco	Haiti	

Table 4.1 : Morphological comparison of *Pterocladiella* species (Continue)

Species / Character	*P. beachiae*[9]	*P. beachiae* (Malaysia)	*P. caerulescens*[10 &11]	*P. caerulescens* (Malaysia)
Height	2.5 cm	up to 3cm	3.3 – 7cm	3cm
Habit	ni	Mostly long pyramidal	ni	Obpyramida at distal part of axis
color	ni	Blackish red to green	Blackish to green	Blackish red to green
Stolon form	Terete to semicompressed	Semicompressed to cylindrical	Subcylendrical to compressed	Cylindrical to semicompressed
stolon width & thick (µm)	ni	150-358/ 123-205	140 -350 / ni	179-312/159-283
Branches form	ligulate	Lanceolate to ligulate	Oblanceolate / ligulate / lanceolate	Oblanceolate to ligulate & lanceolate
Erect axis (base /upper)	taper /semicompress/ flattened	Cylindrical / flatted	Terete / compressed/ flattened	Cylindrical// flattened
Erect axis(width/ thick (µm)	ni	Up to 1411/68 - 85	up to 1800 /40-100	up to 1460/160
Branching	Pinnate to alternate	Irregular , pinnate to unbranched	Simple alternate to quadripinnate	Alternate, distichous to pinnate or irregular
order of branching	up to 3	Mostly 2	3	3
Tetrasporangial sorus	ni	Rounded to obovoid & concave	ni	Long ovoid to rounded
Tetrasporangia sori (LxW)(thick)(µm)	ni	247-1075 x 237- 931/ up to 216	ni	743 -2185 x 300-667/100-140
Tetrasporangia (diam./Lx W) (µm)	ni	12-21 /19-43 x 9-21	Up to 50 diam.	18-25/31- 41 x 20-27
Tetrasporangia arrangement	ni	Irregular	Irregular	Irregular
Tetrasporangial margins	ni	absence	Present	Present
Axis cortical cells in surface view	ni	Long conical to ovoid/ 5-13 x 2-5	ni	Oval to conical, 5-12 x 3-6 µm
Axis cortical cells layers	3-4	2-3	3-4	3-4
Axis outermost cortical cells	Isodiametric to anticlinal	4-15 x 3-7 µm , anticlinal	4-8 x 3-4 µm, anticlinal	5-10 x 3-6 µm , anticlinal
Axis Inner cortical cells	Isodiametric	8-12 x 5-8 µm , periclinal	Up to 15µm, rounded to periclinal	7-13 x 5-11, periclinal
Axis medullary cells	ni	Larger than inner cells,1-3 rows	10-20 µm diam	Smaller than inner cells in 4-5 rows
Stolon cortical cells in surface	ni	Oval to rounded, 7-15 x 3-8 µm	ni	Angular to conical, 7-14 x4-10 µm
Stolon cortical cells layers	3-4	2-3	ni	3 - 4
Stolon outermost cortical cells	ni	5-11 x 3-7µm , anticlinal	ni	7-12 x 4-9 µm , anticlinal
Stolon inner cortical cells	ni	10-16 x 6-10 µm , periclinal	ni	10 -19 x 9 -14 µm , periclinal
Stolon medullary cells	ni	Smaller than inner cells, > layers	ni	Smaller than inner cells, > 10 layers
Rhizines distribution	Abundant in axis &low in stolon	Abundant in erect axis & few in stolon	Abundant in medulla	Abundant in erect axis & few in stolon
rhizoid (height/diameter)	Peg-like	Peg-like	Peg-like	Peg-like
Cystocarp (L x w / thick)	Unequal bilocular	Unequal bilocular, slender to oval, up to 1300 x365 µm / 300µm	Unequal bilocular/elongated	Unequal bilocular, elongated cylindrical up to1382 x 205 µm / 220 µm
Type locality	Costa Rica, Caribbean	Costa Rica, Caribbean	New Caledonia	New Caledonia

Table 4.2: Morphological comparison of *Aphanta* species

species / **Character**	***Aphanta pachyrrhiza*[1]**	***Aphanta sp.* (Malaysia)**
Height	3.5 cm.	0.75 cm.
Color	ni	Reddish to pink
Stolon	Terete / 1mm diameter/ nodes common on stolon	Terete / 250 µm diameter
Attachment systen	Brush-type & peg-like	extensively branched stolon & peg-like rhizoids
Erect axes (width/thick)	1.5-2.8 mm / 400-500µm	0.5 – 1.3 mm/ 50-87µm
Branches	Lanceolate to ligulate/ basally constricted	Ovate , ligulate to lanceolate/ basally constricted
Branching	Up to 3 order/ distichous irregular to pinnate	Rare/ 1 order/ opposite to irregular
Apices	Emarginated apices	Obtuse to mucrunate
Cortical cells in surface view	ni	5.5-8.8 x 3.1–5.6 µm
Cortical cells arrangement	Anticlinally elongate	Isodiameteric
Outermost cortical cells	7.0-9.5 x 4.5-5.5 µm	5.9-8.0 x 4.9-6.3 µm
Inner cortical cells	8.0- 14.5 x 6.0-12.0 µm	9.4-14.0 µm
Medullary cells	7.5-12.0 µm	8.0 -12.5 µm
Rhizines	2.5-4.0 µm/ mostly in inner cortex	4.0-5.5 µm / among medullary cells
Type locality	KwaZulu-Natal, South Africa	

1-Tronchin & Freshwater (2007) (ni: no information)

4.1.2 FAMILY GELIDIACEAE

4.1.2.1 *Gelidium* cf. *crinale* var. *perpusillum* Piccone et Grunow (Figure 4.7)

Piccone 1884: 317; Dawson 1954, p. 421, Figs. 31e-f

Description: Plants dark brown to purplish, small, up to 1.3 cm tall, forming mats on the mangrove roots and pneumatophores, attached to substratum by discoidal form aggregation of rhizoids from a cylindrical stolon. Erect axes and branches arising from stolon, rarely to sometimes sparsely alternately to distichously branched, one order of branching (Figs 4.7A, 4.7B). Erect axes cylindrical with no constriction at point of origin and branching, 72-210 µm in diameter, tapering toward apices and ending in acute apices with dome-shaped apical cell (Figs 4.7C). In surface view of erect axes, cortical cells long ovoid to ovate, 5.0-13.0 x 2.0-7.6 µm, aligned transversely especially in terminal part of erect axes (Figs. 4.7D).

In cross section, erect axes consist of 3 – 4 layers of cortical cells (Fig.4.7E), outermost cells elliptical to rounded, 6.0-11.0 x 5.0 -8.0 µm, mostly arranged periclinally in all layers, inner cortical cells some larger, 7.8-13.3 x 4.8-8.3 µm. Medulla consist of large colorless rounded cells, 10-16 µm in diameter, usually six precentral cells surrounding one axial central cell and one to two layers of smaller medullary cells were observed in outer part of medulla. Rhizines translucent, 3.0 -6.7 µm and aggregated around pericentral cells, mostly at external part of the cells with some rhizines penetrating to inner cortical layer.

Stolon, cylindrical, 100 to 201 µm in diameter, attached in intervals to substratum by conical aggregation of rhizoids with discoid end (Fig. 4.7F); in surface view, ovoid cells, 8.4-16.2 x 3.6-8.2 µm, arranged transversely (Fig. 4.7G); in cross section, stolon consisting of 3-4 layers of preclinal layers of cortical cells; outermost cortical cells

elliptical, 6.1-12.5 x 3.9-6.6 µm and medulla composed of one axial and six pericentral large rounded cells, 12.0-16.5 µm in diameter (Fig. 4.7H); rhizines number lower than erect axes and usually distributed in outer part of medulla and rarely among medullary cells, mostly mixed with inner layers of cortex cells (Fig. 4.7H) .

Tetrasporangial sori on the apices of erect axis and branches, tetrasporania irregularly disposed in a cone-shaped to cylindrical tetrasporangial stichidia (Fig. 4.7I), 224-995 x 121-404 µm.Tetrasporangia disposed irregularly (Fig. 4.7J). cruciately or tetrahedrally divided , rounded in surface view, 8-31 µm in diameter and 12-40 x 10-24µm in transverse section (Fig. 4.7K).

Cystocarps equally bilocular, subspherical, 338-522 x 424-560 µm, with or without sterile margin and open by ostioles on two sides (Fig. 4.7L). Transverse section through the cystocarp showed a transverse chain of placenta which carpospores were produced on their both sides (Fig. 4.7M). Carpospores long ovoid 30-50 µm in length. Spermatangial stichidia in appendage forms, near the cystocarps (Fig. 4.7N); spermatangial sori consisting of rounded to ovoid small spermatia, 1.5-2-4 x1.4-2.4 µm, supperfacially locted among cortical cells (Fig. 4.7N).

Ecology: Plants mostly are epiphytic on roots, pneumatophores and basal parts of mangroves trunks which mostly grow near the rocky shores of intertidal fringes, and their holdfasts are tightly attached to the bark of mangroves roots and peneumatophores and provide habitat for other red algae such as *Catenella*, *Bostrychia* and *Caloglossa* species which are common red algae in mangrove ecosystems.

Global Distribution: Ethiopia, Indian Ocean Islands, Indonesia, Philippines, South Pacific (Fiji), Australia (Queensland).

Local distribution Malaysia: Port Dickson, Negeri Sembilan (2°24' 54" N; 101°51' 10" E), 30 Dec.2009, J. Sohrabipoor, PSM12503, 28 Feb, 2010, PSM122517, 2.Aug, 2011, PSM12577; Pulau Besar (2°,06,' 57" N 102°, 19' ,54" E), Melaka, 11 Apr. 2010, J. Sohrabipoor, PSM12522 (slide); Kampung Dandulite (5°59 '43.45" N / 117°54 ' 50.70 " E) Sandakan, Sabah, 9. Nov. 2010, J. Sohrabipoor, PSM12554; Teluk Kemang, Port Dickson (2°26 ' 38 " N/ 101°51 ' 21 " E), 29 Aug. 2011, J. Sohrabipoor, PSM12590, 11 Nov. 2011, PSM12609.

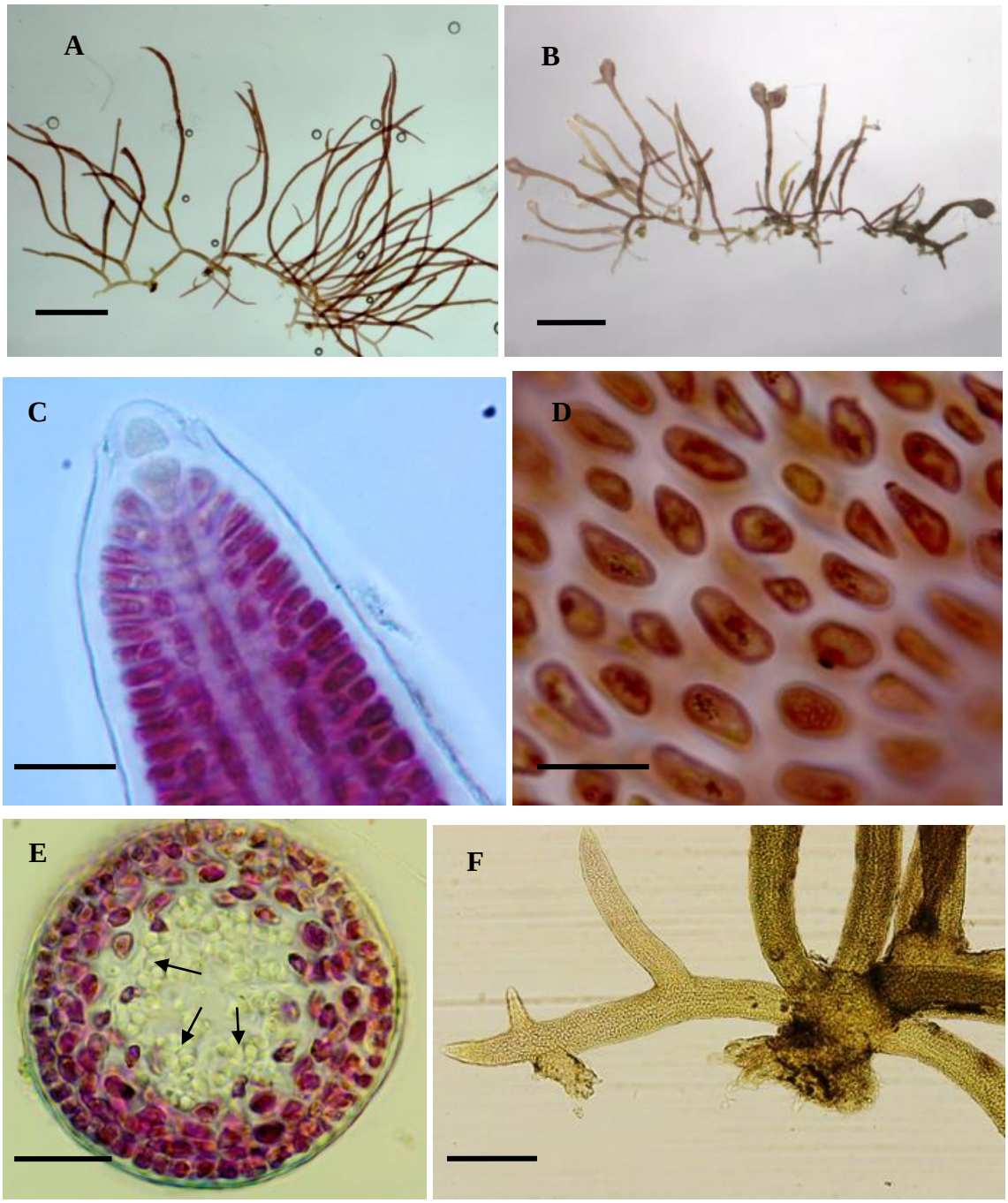

Figure 4.7: *Gelidium* cf. *crinale* var. *perpusillum.*

(A) Habit of plant. Scale=2mm, (B) Habit of carposporophyte plant. Scale=2mm, (C) Dome-shaped apical cell. Scale bar= 50µm, (D) Cortical cells in surface view of middle parts of erect branches with transverse arrangement. Scale bar=20 µm, (E) Transverse section of middle part of erect branch shows isodiamtric to preclinal arrangement of cortical cells and distribution of rhizines (arrows). Scale bar=50 µm, (F) Stolon and discoid holdfast. Scale bar=200 µm.

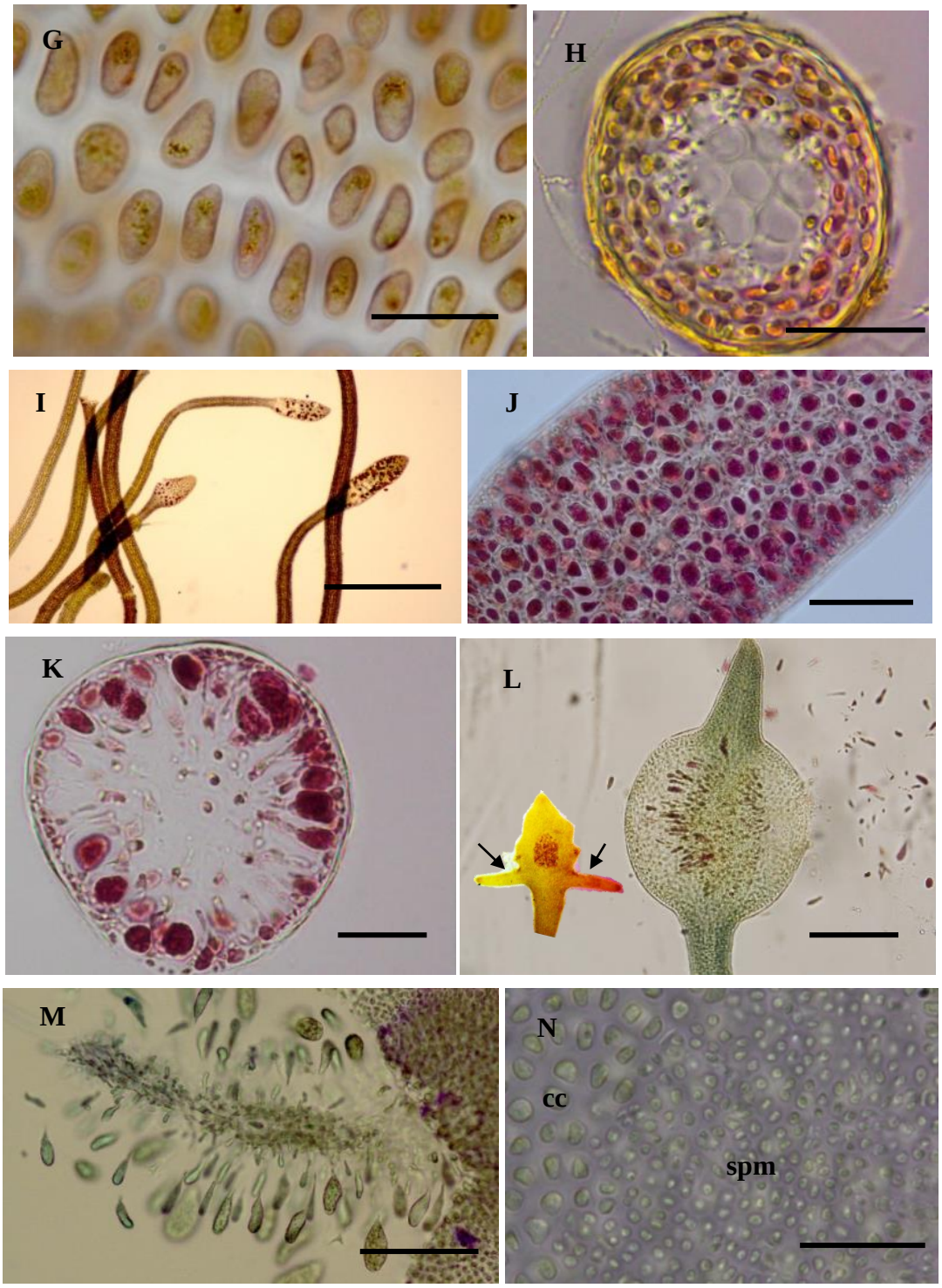

Figure 4.7: *Gelidium* cf. *crinale* var. *perpusillum.*

(G) Cortical cells in surface view of stolon with transvers arrangement. Scale bar=20 µm, **(H)** Transverse section of stolon shows preclinal arrangement of cortical cells and distribution of rhizines in outer medulla. Scale bar=50 µm, **(I)** Terminal conical to slender tetrasporangial stichidia. Scale bar=500 µm, **(J)** Surface view of tetrasporangial sorus with irregular arrangement of tetrasporangia. Scale bar=200 µm, **(K)** Transverse section of tetrasporangial stichidia. Scale bar=50 µm, **(L)** Bilocular globose cystocarp and spermatangia stichidia near the cystocarp base (arrows). Scale bar 100 µm, **(M)** Transverse section of cystocarp. Scale bar= 20 µm, **(N)** Spermatangial sorus (spm) among cortical cells (cc), Scale bar= 20 µm.

4.1.2.2 *Gelidium* sp. nov. 1. (Figures 4.8)

Thalli reddish to purple, forming tufts composed of creeping stolon and erect axes, up to 1.3 cm height; attached to the substratum by brush-like haptera or discoid holdfast. Creeping axes or stolon cylindrical, 71-375µm in diameter; erect axes cylindrical at base, compressed and flattened at middle and upper part, up to 1.6 mm wide and 102 µm thick, sometimes with undulate margin; branching irregular, rare to polytrichous in vegetative thalli and abundantly proliferated in reproductive phase, up to six order of branching in reproductive phase; apices obtuse to acute with prism, rounded to dome-shaped apical cells. Tetrasporangial stichidia were borne on terminal part of ultimate branches, sometimes in a palmate aggregation ; tetrasporangial sori oval to irregular in shape, 436-1251 x 204-936 µm; tetrasprangia cruciately divided and arranged irregularly; sori with no sterile margins.Tetraspores rounded in surface view, 13-30 µm in diameter. Cystocarp equal bilocular, solitary or grouped together at end of branches in palmate form, oval-shaped to globose, marginated, with one ostiole on each side. Spermatia in small patches among the cortical cells of pericarp.

Holotype : Batu Feringhi 3, Pulau Pinang (5 ° 28' 51" N; 100 ° 15' 15" E), 8. Sep. 2009, J. Sohrabipoor, PSM12493.

Isotypes: Batu Feringhi 3, Pulau Pinang (5 ° 28' 51" N; 100 ° 15' 15" E), 8. Sep. 2009, J. Sohrabipoor, PSM12494.

Type locality: Batu Feringhi 3, Pulau Pinang (5 ° 28' 51" N; 100 ° 15' 15" E), Malaysia

Ecology: plants grow on the rock in the intertidal regions exposed to the wave action and make a purplish turf on the rocks.

Specimens examined: Holotype, Pulau Pinang (5 ° 28' 51" N; 100 ° 15' 15" E), Malaysia, 8. Sep. 2009, J. Sohrabipoor, PSM12493; PSM12494; Pulau Pangkor (4˚, 13,' 35" N 100˚, 32', 29" E) Sitiawan, Malaysia, 28. Apr. 2010, J. Sohrabipoor, PSM12524; Kem Bina Negara (5˚, 18,' 0.52" N /100˚, 11', 4" E) Pulau Pinang, 6. Apr. 2012, R. Rabiei & J. Sohrabipoor, PSM12630, PSM12631, PSM12632; Pantai Bukit Keluang (5° 48' 5.84" N; 102 ° 36' 17" E), Kuala Terengganu, 17.Apr.2012, J. Sohrabipoor, PSM12641.

Description: Plants of the species grow as extensive turfs or small clumps on the rocks exposed to the wave action in the intertidal zones or sometimes in the highly sloped area in the upper part of the intertidal zones. Plant consists of a cylindrical stolon and prostrate branches and flattened erect axis. Erect axes basally cylindrical or cuneate and sometimes abruptly flattened (Figs. 4.8A -C) , up to 1.3 mm high and from 54 to 332 µm at basal portion to 1.6mm wide in middle and upper flattened parts and up to 101 µm in thick. Erect axes simple and unbranched to opposite, polytrichous or abundantly branched, especially in upper parts of reproductive plant which produce cylindrical proliferated branches (Figs. 4.8D-E). The axis and branches were long lanceolate, ligulate to linear. Base of branches cuneate and their apices are obtuse or acute with dome-shaped to obpyramidal apical cells (Fig. 4.8F). Cortical cells in surface view of erect axes and branches ovoid to rectangle, 4.8-10.5 x 3.4-6.6 µm, aligned irregularly to longitudinally (Fig. 4.8G). In cross section, erect axes and branches consist of 3-4 layers of cortical cells and medulla, cortical cells 5.5-8.2 µm in diameter, mostly isodiametric; medullary cells larger, 13 to 20 µm in diameter, rhizines abundant among medullary cells, 2.5 to 6.5µm (Fig. 4.8H). Creeping axes and stolons cylindrical, 71 to 375 µm in diameter, attached to substratum by discoid holdfast and brush-like rhizoids (Figs. 4.8I & 4.8J). Cortical cells in surface view of stolon were

polygonal to ovoid 7.3-16.2 x 4.0-8.8 µm, mostly aligned transversely to irregularly (Fig. 4.8K); in transverse section of stolon outermost cortical cells were long ovoid, 5.8-10 µm in length and aligned anticlinally (Figs. 4.8L & 4.8M), medulla consist of larger rounded cells, rhizines sparse compared to the erect branches and mostly distributed in external layers of medullary cells.

Tetrasporangial stichidia in oval, rounded to irregular shape were produced on ultimate stichidial branchlets, 436.0-1251 x 204-936 µm, and 137-210 µm in thick, mostly in palmate groups (Fig. 4.8N), tetrasporangia disposed irregularly, cruciately or tetrahedraly divided; in surface view of tetrasporangial sori, tetrasporangia, rounded to oval-shaped, 19.0-30.7 µm in diameter and 25.6-48 x 14.6-31.2 µm in transverse section (Fig. 4.8O).

In carposporophyte plant, swollen cystocarps, in spherical to oval forms were located on ultimate branchlets, mostly in palmate groups, 171-697 x 160-453 µm (Fig. 4.8P). In transverse section of cystocarp, a chain of placenta cores were located transversely and produced carpospores on both sides (Fig. 4.8Q); carpospores long ovoid to ovoid, 25.2-50.1 x 11.1-19.2 µm. Spermatangial sori were observed on the cystocarp wall (pericarp) among cortical cells (Fig. 4.8R).

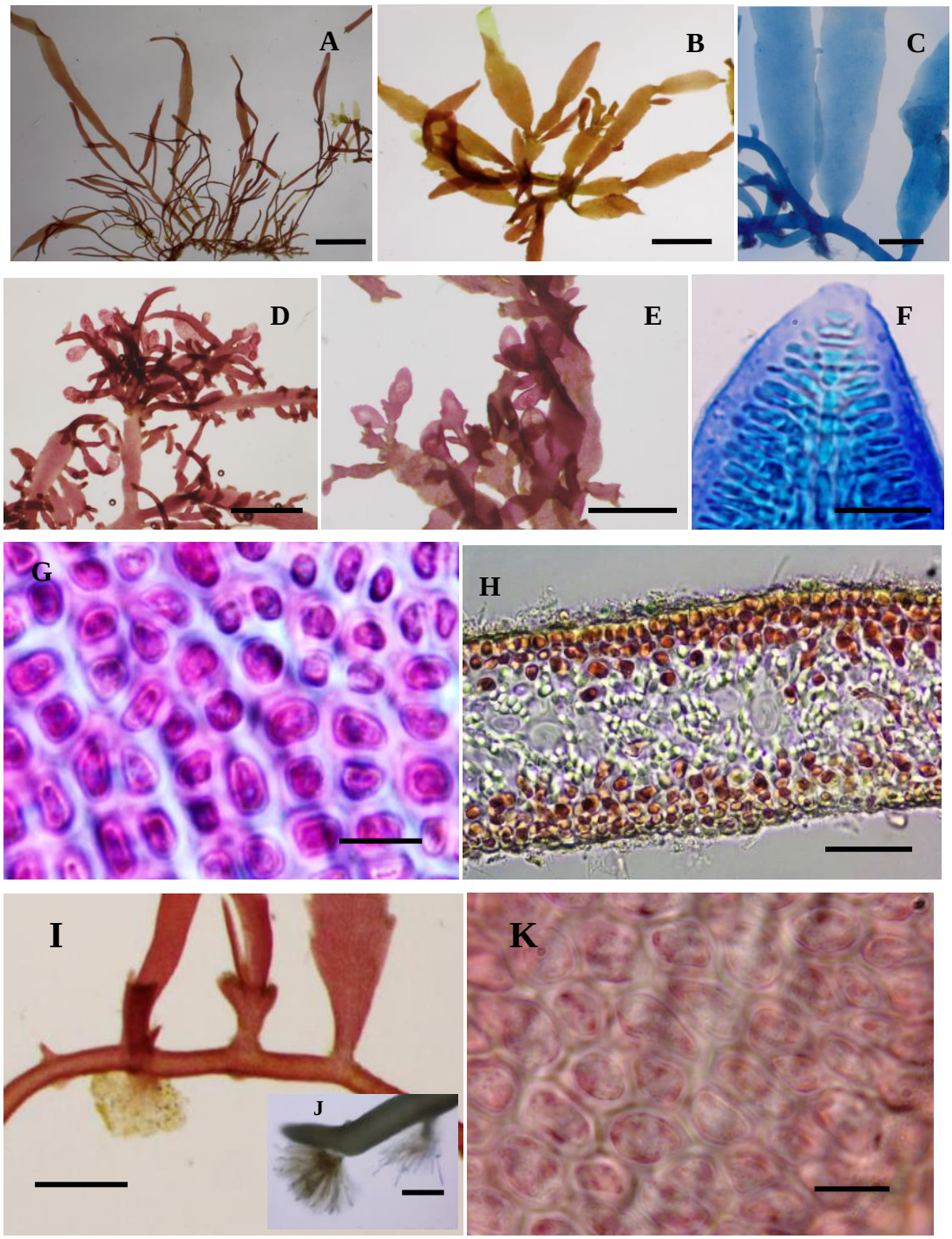

Figure 4.8: *Gelidium* sp. nov.1

(A) Habit of plant from Pulau Pinang. Scale bar =2mm, **(B)** Habit of plant from Kuala Terengganu. Scale bar =1mm, **(C)** Abruptly flattened erect axes with short cylindrical basal part. Scale bar=1 mm, **(D)** Habit of terasporophyte plant from Pulau Pinang. Scale bar =2 mm, **(E)** Habit of carposporophyte plant from Pulau Pinang. Scale bar =1 µm, **(F)** Obpyramidal apical cell. Scale bar= 50µm, **(G)** Cortical cells in surface view of erect branches with irregular arrangement. Scale bar=10 µm, **(H)** Transverse section of erect branch shows isodiametric cortical cells, rounded medullary cell and rhizines. Scale bar=40 µm, **(I)** Stolon with discoid and brush-like holdfast. Scale bar=200 µm, **(J)** Terminal part of stolon with brush-like rhizoids. Scale bar=200 µm, **(K)** Cortical cells in surface view of stolon. Scale bar=10 µm.

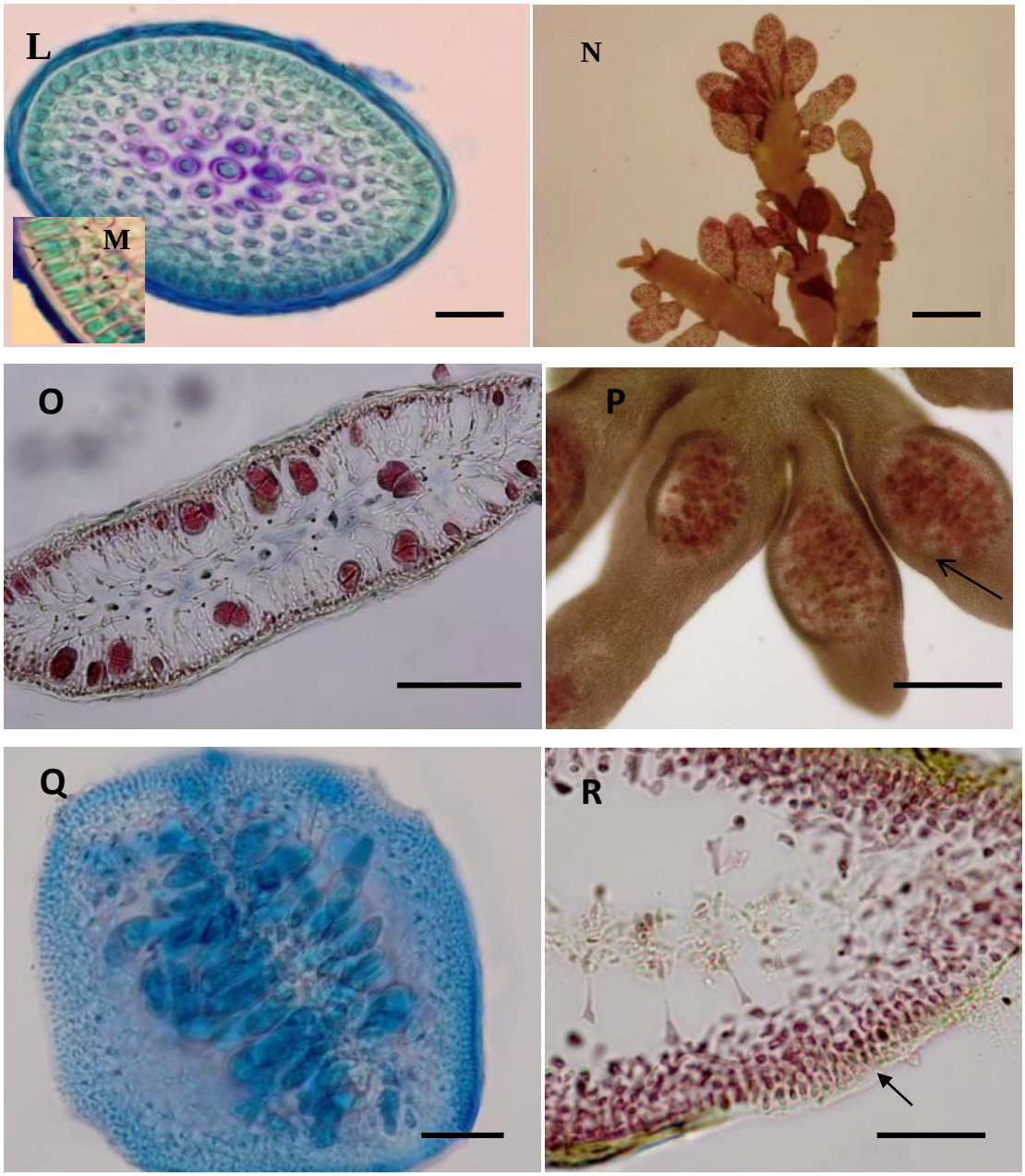

Figure 4.8: *Gelidium* sp. nov.1

(L) Transverse section stolon shows anticlinall arrangement of cortical cells and low number of rhizines among medullary cells. Scale bar=40 µm, **(M)** Anticinal arrangement of cortical cells in transverse section of stolon, **(N)** Tetrasporangial stichidia in palmate form on terminal branches. Scale bar=500 µm, **(O)** Transverse section of tetrasporangial stichidia. Scale bar=100 µm, **(P)** Carposporangial stichidia at the terminal part of branches. Scale bar 200 µm, **(Q)** Trasverse section of cystocarp. Scale bar= 50 µm, **(R)** Trasverse section of cystocarp show spermatangial sorus (arrow).Scale bar= 40µm.

4.1.2.3 *Gelidium* sp. nov. 2 (Figure. 4.9)

Thalli small, cartilaginous, caespitose, most axis and branches decumbent, brownish to reddish purple in color, up to 6 mm height; creeping axes cylindrical to subcylindrical, 131-234 µm diameter; attached to substratum by irregularly disposed short discoid holdfasts or stalked holdfast; short stiff erect branches arising from creeping axes, cylindrical basally to semicompressed in middle and reproductive upper parts; from 177 to 237 µm at basal part to 307 µm broad and 66 to 150 µm thick in upper parts; irregularly to rarely distichously branched, mostly in divaricate and at right angle, with alternately to opposite disposed pinnulae; apex acute or rounded with convex, obpyramidal to dome-shaped apical cells . In cross section, branches consist of 3-5 layers of cortical cells and rounded large medullary cells; outermost cortical cells ovate to elliptical, 5-9 x 4-7 µm and medullary cells 15-22µm in diameter; rhizines few, 3-5 µm, scattered in the outer part of medulla.

Tetrasporangial sori on the semicompressed apex of ramuli, long oval, obovate, to irregular in shape, 290-816 x 127-142 µm, without sterile margin, tetrasporangia roundish or ovate in surface view, 13-20 µm in diameter and cruciately divided.

Cystocarps on the terminal parts of ramuli, unequal or equal bilocular, 404-575 x 351-414 µm, with or without sterile margin, with a single ostiole on each side or sometimes in unequal bilocular cystocarps only at the swollen side. Spermatangial sori scattered or in patches on spermatangial stichidia near the cystocarps, spermatia small, rounded to ovoid, 2.3-3.6 x 1.4-2.8 µm.

Ecology: growing in dense clumps on upper intertidal rocks as epizoic on limpets and shells.

Holotype: PSM12589-1 (Fig. 27), coll. J. Sohrabipoor, 29 Aug. 2011.

Type locality: Teluk Kemang, Port Dickson, Negeri Sembilan (2 ° 26' 38" N; 101 ° 51' 21" E), Malaysia.

Specimens examined: Holotype, Teluk Kemang, Port Dickson, Negeri Sembilan (2 ° 26' 38" N; 101 ° 51' 21" E), Malaysia, 29 Aug. 2011; J. Sohrabipoor, PSM12589-1 & PSM12605; Panti Bukit Keluang, Kuala Terengganu (5 ° 48' 5.84" N; 102 ° 36' 17" E), Malaysia, 17 Apr.2012, J. Sohrabipoor, PSM12642, PSM12646.

Description: Thalli formed thin mats and were epizoic on the population of small molluscs and limpets attached to the rocks or artificial cement blocks in the intertidal regions, up to 6 mm tall. Individual plants are generally difficult to distinguish. Brownish to reddish in color (Figs. 4.9A-C). Most branches are decumbent and laterally growing from conspicuous or inconspicuous stolons. Erect branches are small and mostly terminated to reproductive structures (Figs. 4.9A-C) and arising irregularly from the upper side of the decumbent branches; basally terete and cylindrical and becoming slightly semicompressed and wider in middle and upper portions; 72-210 µm in diameter; branched irregularly alternate to subdistichously at wider semicompressed parts. The branches gradually taper to acute or obtuse apices with convex, obpyramidal to dome-shaped apical cells (Figs. 4.9D & 4.9E). Cortical cells in surface view of the erect axes are uniform (Fig. 4.9F) mostly rounded to ovoid, 4-10 x 3-7 µm. In cross section, erect axis and decumbent branches were similar, consisting of 3-5 layers of

cortical cells and large rounded medullary cells (Figs. 4.9G & 4.9K). Outermost cortical cells in erect branches and decumbent branches and stolons were similar in size, 6.6-11 x 5-8.5 µm but their arrangement in erect branches were anticlinal while in the decumbent branches were preclinal (Figs. 4.9G & 4.9K), medullary cells also of same size, 15-22 µm and rounded in shape, rhizine few and scattered in outer layers of medullary cells (Figs. 4.9G & 4.9K).

The decumbent branches were terete to semicompressed, up to 234 µm in diameter and strongly attached to substratum by short coalescent rhizoids with discoid holdfast; holdfast up to 515 µm in diameter, and sometimes stalked holdfast observed near the apices of decumbent branches (Figs. 4.9H & 4.9I). Cortical cells in surface view of the stolon and decumbent branches are larger than erect branches, 7-13 x 4-9 µm and were irregularly arranged (Fig. 4.9J).

Tetrasporangial stichidia were borne on terminal part of erect branches and lateral branches; ovoid, long ovoid or irregular in shape (Figs. 4.9B & 4.9C), 283-1904 x 290-434 µm and 127 to 142 µm thick; tetrasporangia disposed irregularly (Fig. 4.9L), rounded to oval-shaped, 13 to 20 µm diameter in surface view and 23-49 x 18-34 µm in cross section (Fig. 4.9M) . A mixture of young sporangia and matured tetrasporangia were observed in surface view and cross section of tetrasporangial stichidia (Figs. 4.9L & 4.9M).

Gametophyte thalli were monoecious, cystocarps were globose with two equal locules and one ostiole on each side or unequal bilocular in oval-shaped swollen cystocarps with one ostiole in elevated swollen side (Figs. 4.9N & 4.9O). Carpospores were borne on the successive gonimoblasts which were transversely (Fig. 4.9O) located

in cross section of cystocarps, dumbbell- shaped with long attenuated end, 19.5-52 x 9-18 µm, released in large number from the large ostioles. Spermatangial sori sparsely or in patch form, borne on special stichidia near the base of cystocarps or on the nearest branch to the cystocarp (Figs. 4.9P). Spermatangia were small, 2.3-3.5 x 1.4-2.4 µm, and observed in rounded to ovoid- shape among superficial cortical cells (Fig. 4.9Q).

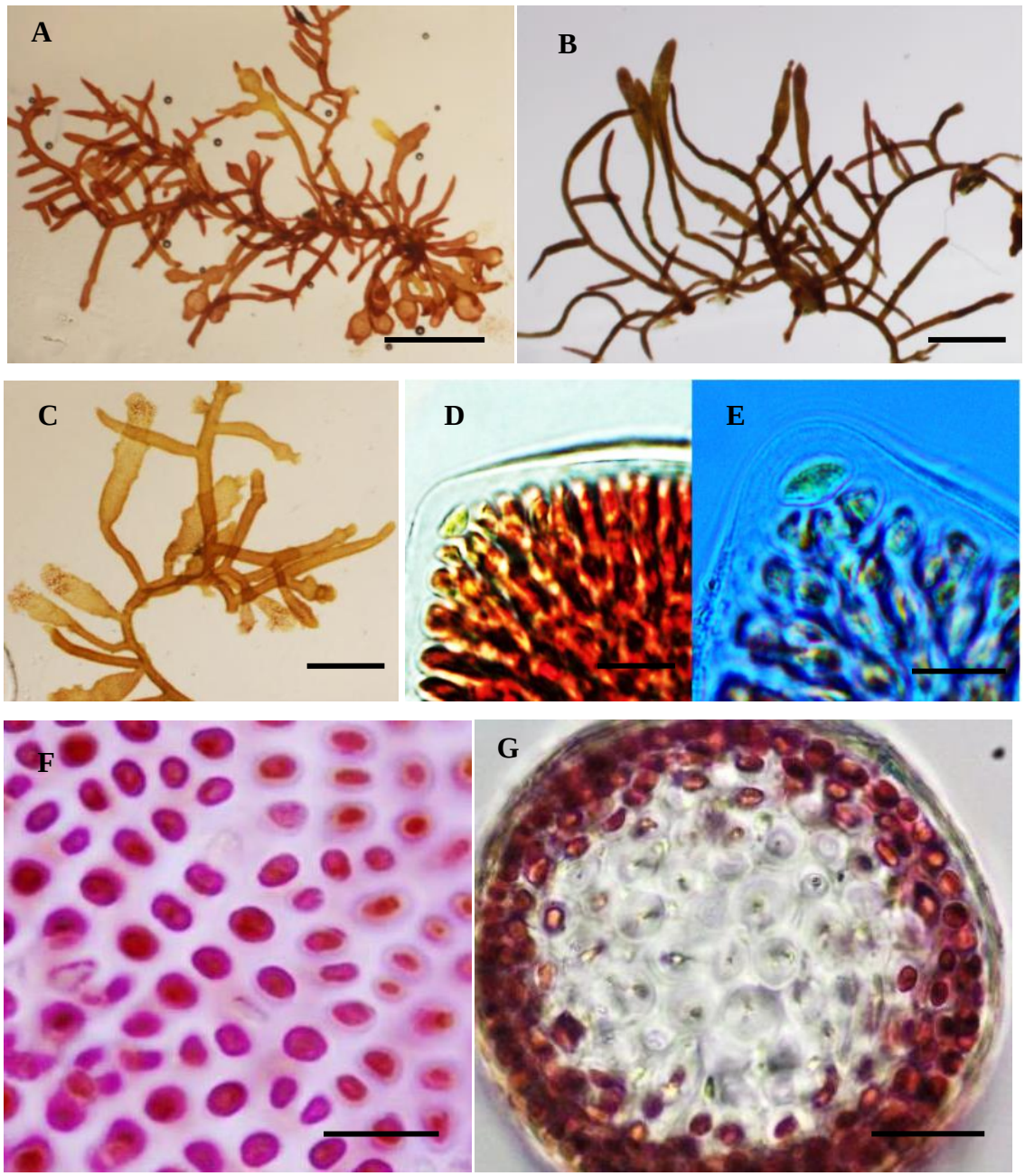

Figure 4.9: *Gelidium* sp. nov. 2

(A) Habit of carposporophyte thalli (Holotype). Scale bar =1mm, **(B)** Habit of terasporophyte thalli from Kuala Terengganu. Scale = 1mm, **(C)** Habit of terasporophyte thalli from Teluk Kemang. Scale=1mm, **(D)** Triangular apical cell. Scale bar =10µm, **(E)** Lenticel apical cell. Scale bar = 10µm, **(F)** Cortical cells in surface view of erect branches with irregular arrangement. Scale bar=10 µm, **(G)** Transverse section of erect branch shows preclinal arrangement of cortical cells and rounded medullary cell and low number of rhizines. Scale bar =40 µm.

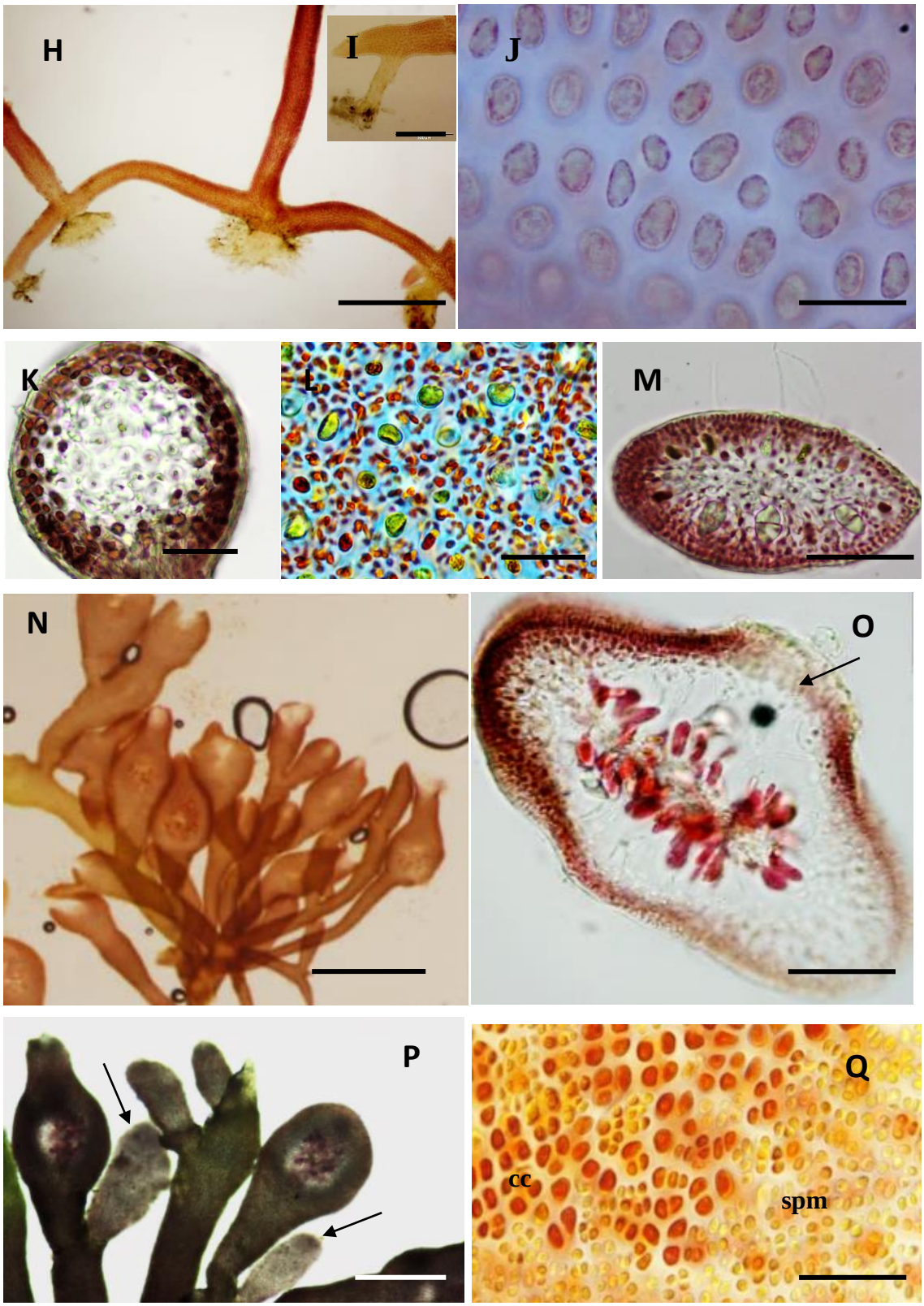

Figure 4.9: ***Gelidium*** **sp. nov. 2**

(H) Stolon and discoid holdfast. Scale bar =500 µm, (I) Terminal part of Stolon with peg-like holdfast. Scale bar =200 µm. (J) Surface view of stolon cortical cells. Scale bar =500 µm, (K) Transverse section stolon with preclinal cortical cells and rare rhizines. Scale bar=50 µm, (L) Surface view of tetrasporangial sorus with irregular arrangement of matured and young tetrasporangia. Scale bar=100 µm, (M) Transverse section of tetrasporangial stichidia. Scale bar =100 µm, (N) Large cystocarp at the terminal part of branches. Scale bar = 1mm, (O) Trasverse section of cystocarp shows unequal bilocular form with large ostiole. Scale bar= 50µm, (P) Spermatangial stichidia near the cystocarp (arrows). Scale bar =500 µm, (Q) Spermatangial sorus (spm) among cortical cells (cc). Scale bar =20 µm.

Table 4.3: Morphological comparison of *Gelidium* species.

Species characters	***Gelidium sp. nov. 1* (Malaysia)**	***G. usmanghanii*** 1	***G. divaricatum*** [2, 3]	***Gelidium* sp. nov.2 (Malaysia)**	***Gelidium crinale*** [4&5]	***Gelidium cf. crinale var. perpusillum* (Malaysia)**
Height	Up to 13mm	Up to 7cm	Up to 20 mm	Up to 6 mm	Up to 7cm	Up to 13mm
Habit	turfy	Turf - forming	Mat form	Mat form	Turfy and epilitic	Mat form on mangrove roots
Color	Reddish to purple	ni	Brownish to purple	Brownish to reddish	Dark red-brown to yellowish	Blackish to purple
Stolon	Cylindrical, 71-375 µm diam.	Cylindrical, 0.3-0.5mm in diam.	teret to compressed	Cylindrical to semicompressed	Cylindrical/ 100- 200 µm diam.	cylindrical
Erect axes and branches	Cylindrical at base, gradually to abruptly flattened above, up to 1.6mm width	teret at base, abruptly turning to flat and spiral frond, 1-3mm width	Cylindrical to subcylindrical, 400-700 µm	Terete to subterete 106 -387 µm	Cylindrical , flattened at apex up to 350µm width, ,	Cylindrical / 72-210 µm
Branching	Irregular , rare to polytrichous in reproductive stage	Unbranched to sparse to densely branched in margin of frond	Alternately to bipinnate	irregular to subdistichous	Distantly and irregular	Rare / alternate to distichous
Cortex layers	3-4 layers	ni	4 layers	3-5 layers	3-4 layers	3-5 layers
Outermost cortical cells	Rounded, 5-8 µm In diam.	ni	Ovoid,6-10 x 4-6 µm anticlinal	Ovoid to elliptical/ 5-8 x 4-7	rounded/ anticlinal/up to 6 µm diam.	Elliptical / periclinal /6- 11 x 5-8
Inner cortical cells	Rounded, same size as outer cells	ni	Lobed / 6-14 µm in diam.	Elliptical to rounded	Larger than cortical cells, up to 10 µm	Elliptical
Medulla	Rounded, 13-20 µm	ni	With 3-4 side arms/ sparse	Rounded/ 15-22 µm	3-6 cells across	Rounded/ 10-16 µm
Rhizines distribution	Abundant in medulla of erect axes and rare in stolon	Abundant below the cortex	Outer medulla	Few in outer medulla	Throughout in medulla in young branches and outer medulla in older branches	Abundant around medullary cells
Tetrasporangial sorus	In swollen ovoid to irregular stichidia mostly in palmate groups on apex of branches	Stichidia in different shape, branched or unbranched	Ovoid to spherical	Semicompressed/ 283-1904 x 290-434 / 127 -142µm thick	terminally in compressed Stichidia, sometimes furcate, intercalary, 0.5-2mm x 200-500 µm	Cone-shaped stichidia at apex of branches / 223-995 x 121-551 µm
tetrasporangia	19-31 µm diam , 25-48 x14-31 µm in cross section	Up to 45 x 34 µm	40-50 x 28-40 µm	13-21 µm diameter/ 23-49 x 18-34 µm in cross section	Ovoid, up to 30 µm in diam.	8-17 µm diameter/ 12-39 x 10-24 µm in cross section
Spermatangial stichidia	In patches on the cystocarp wall	ni	ni	Near cystocarp/ with patches of sori	ni	Near cystocarp/ with patches of sori
Cystocarp	Bilocular, in palmate groups on apex of branches	Bilocular/ in branched ramuli	Terminal / swollen to globose /450-65 µm 0 in diam.	Equal bilocular or unequal bilocular/ marinate/ 252-575 x229-469 µm/ 136-147 µm thick	Subterminal in clustered branchlets/500-600 µm across	Equal bilocular or unequal bilocular/ marinate or no margin/ 388-522 x 424-560 µm
Type locality	Pulau Pinang	Karachi, Pakistan	Hong Kong	Teluk Kemang, Malaysia	England	

1. Millar & Freshwater, 2005; 2. Xia *et al.* 2002; 3. Lee 1994; 4. Feldmann & Hamel, 1936; 5. Santelices 1977.

4.1.3 FAMILY GELIDIELLACEAE

4.1.3.1 *Gelidiella acerosa* (Forsskål) Feldmann *et* Hamel (Figure 4.10)

Basionym: *Fucus acerosus* Forsskål, Flor Aegyptiaco-arabica: Post mortem auctoris edidit Carsten Niebuhr: 190, 1775.

Synonym: *Echinocaulon acerosus* (Forsskål) Børgesen, 1932, p. 5.

Ref: Feldmann & Hamel, 1934, p. 533; Zhang & Xia, 1988, p. 21, Figure 1, Pl. I: 9; Xia *et al.*, 2004, p.202, Figs 4-8; Santelices, 1977, p. 5, Fig. 1A-C.

Description: Thallus up to 5 cm high (Fig. 4.10A), blackish to olive green in colour, forming mats on the rocks and corals; creeping and erect axes cartilaginous, semi-rigid. Erect axes are cylindrical to semicompressed, 600-1038 µm in width, arising from entangled creeping or arched stolon; axes branching in one to three orders, primary branching was alternate, unilateral or sparsely irregular and secondary branching was alternate to subopposite and generally short (Figs. 4.10A & 4.10B); sometimes basal branches were ligulate (Fig. 4.10A), ultimate branches were more or less in same size; 297-664 µm in width, no constriction at the base of branches, branches slightly taperin upward and end in acute to obtuse apices with distinct prominent apical cell.

In surface view of erect branches, rounded small cortical cells, 3.1-5.3 µm, were isodiametric and mixed with brownish rounded basal cells of hairs (Fig. 4.10D). In transverse section, erect axes composed of 2-3 layers anticlinally arranged of cortical cells gradually becoming larger inwardly to densely packed rounded to oval-shaped medullary cells, 20-25 x 10-12.5 µm; no rhizine was observed (Fig. 4.10E).

Tetrasporangial stichidia born on the terminal part of the ultimate branchlets with a short basal pedicel and form a slightly swollen yellowish cylindrical stichidia (Figs.

4.10B & 4.10C), 716-1500 x 315-44 6 µm, without strile margin. Tetrasporangia developed acropetally and disposed irregularly under cortex cells; 38-50 µm in length (Figs. 4.10C & 4.10F).

Ecology: Growing on intertidal or subtidal dead coral and crevice of rocks.

Global Distribution*:* Common in the tropics and subtropical seas.

Local distribution in Malaysia: Cape Ruchado, Port Dickson (2˚24 ' 54" N / 101˚51 ' 10 " E), 30 Dec. 2009, J.Sohrabipoor, PSM12500; 2 Aug. 2011, PSM12584; 29 Feb.2010, J. Sohrabipoor, PSM12513; Teluk Kemang (2º 24' 54" N; 101º 51'20" E) , Port Dickson, 28 Feb. 2010, J. Sohrabipoor, PSM12514; Pantai Bukit Kelang- (5 ° 48' 5.84" N; 102 ° 36' 17" E), Kuala Terrenganu , 16 Feb. 2012, J. Sohrabipoor, PSM12640.

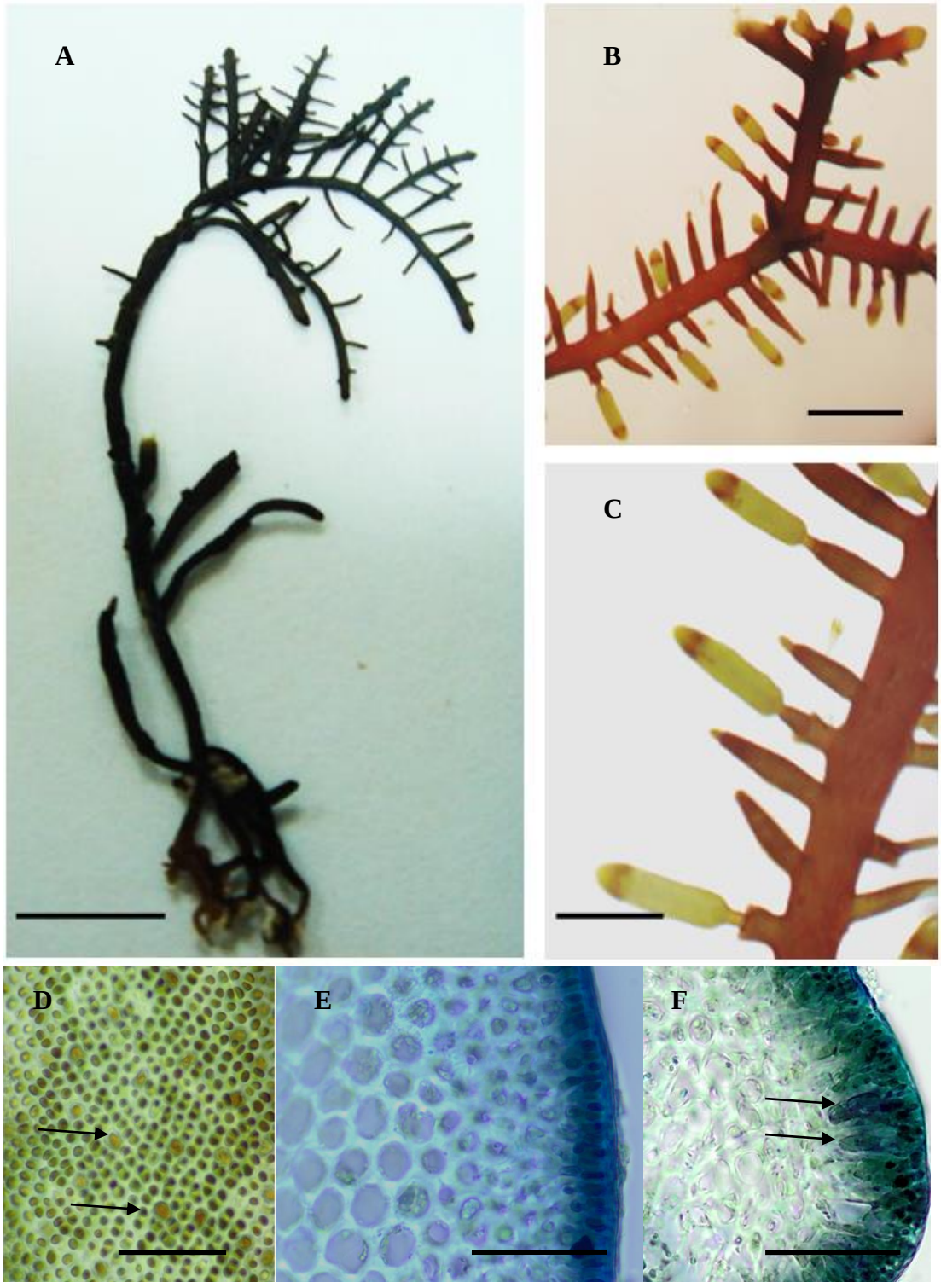

Figure 4.10: *Gelidiella acerosa* (Forsskål) Feldmann et Hamel

(A) Habit of plant. Scale bar=5mm, (B) Habit of tetrasporophyte thalli. Scale=2mm, (C) Pedicellate tetrasporangia stichidia with acropetal development of tetrasporangia. Scale bar= 1mm, (D) Cortical cells in surface view of erect branches with brownish basal cells of hairs. Scale bar=40 µm, (E) Transverse section of erect branch shows anticlinal arrangement of cortical cells (arrow). Scale bar=40 µm, (F) Transverse section of tetrasporangial stichidia, shows position of tetrasporangia (arrows) under cortical cells. Scale bar=40 µm.

4.1.3.2 *Parviphycus sp*. 1 (Figures 4.11)

Desription: Thalli small, creeping on rock surfaces, purplish to olive green in color, up to 1cm tall, composed of prostrate cylindrical filament, 62-104µm in diameter, attached along intervals by holdfast of fasciculate aggregation of unicellular rhizoids (Figs.4.11A & 4.11B). Rhizoids with 2-3 rows of cells at basal part, up to 388 µm in length and 8 µm width (Figs. 4.11C). Erect axes slightly semicompressed to cylindrical, rising from dorsal suface of creeping axes opposite to the fasciculated rhizoids (Figs. 4.11A & 4.11B), mostly unbranched or rarely, sparsely or unilaterally branched; 72-122 µm in diameter, up to 1cm tall. No basal constriction at the base of erect axes and branches; branches terminated in acute apices with prominent dome-shaped apical cell with distichous division in subapical cells (Fig. 4.11D). In surface view of erect branches, quadrate, triangular to oval-shaped cortical cells, 4.7-8.8 x 2.3-6.4 µm, were aligned transversely to irregularly (Fig. 4.11E). Cortical cells in surface view of stolon, long ovoid, 7.5-13.1 x 3-6.5 µm, and arranged transversely (Fig. 4.11F). In transverse section, erect axes composed of 2-3 layers of small elliptical cortical cells mostly same size in all layers, 4.3-7 x 2.5-3.8 µm, aligned preclinally; medulla cosist of one row of axial elliptical cells, up to 15 µm in length and 12 µm in width, with two rows preaxial, large rounded to oval colorless cells (Fig. 4.11G), up to 22 µm in length and 15 µm in width, with clear pit connection between the subsequent cells in longituidinal section (Figs. 4.11H). No rhizine was observed in transverse section.

Tetrasporangial stichidia terminally (Fig. 4.I), distichously (Fig. 4.11J) to unilaterally (Fig. 4.11K) located on the normal erect axes and branches, stichidia up to 673 µm in length, 166 µm in width and 82 µm in thick. Tetrasporangia disposed in open V-shaped regular rows with 4-6 tetrasporangia per row in the large preaxial cells under

cortial cell layers, up to 25 µm diameter in surface view and up to 31 µm in transverse section (Fig. 4.11L) and developed acropetally (Fig. 411.k).

Ecology: Plant usually grows on the surface of rocks mostly near the upper part of the intertidal zone in pale pink color, velvet mat and sometimes among population of *Gelidium* cf. *crinale* var. *perpusillum* on mangrove roots and pneumatophore.

Local Distribution in Malaysia: Cape Ruchado (2˚24 ' 54" N / 101˚51 ' 10 " E), Port Dickson, 20 June 2010, J.Sohrabipoor, PSM12534; Teluk Kemang (2º 24 ' 54" N; 101º 51’20" E), Port Dickson, 2 Aug. 2011, J. Sohrabipoor, PSM12579; Teluk Kemang (2º 24' 54" N; 101º 51’20" E), Port Dickson, 29 Aug.2011, J. Sohrabipoor, PSM12594; 29 Dec.2011, J. Sohrabipoor, PSM12612; Panti Chendering (5° 16' 9.63" N; 103° 11' 18.62" E), Kuala Terengganu, 17 Feb. 2012, J. Sohrabipoor, PSM12647; Pulau Manukan (5˚ 32' 27.68 " N, 116˚ 00 '11.34 " E) Kota Kinabalu, Sabah, 3 Aug. 2012, J. Sohrabipoor & R. Rabiei, PSM12674, PSM12675.

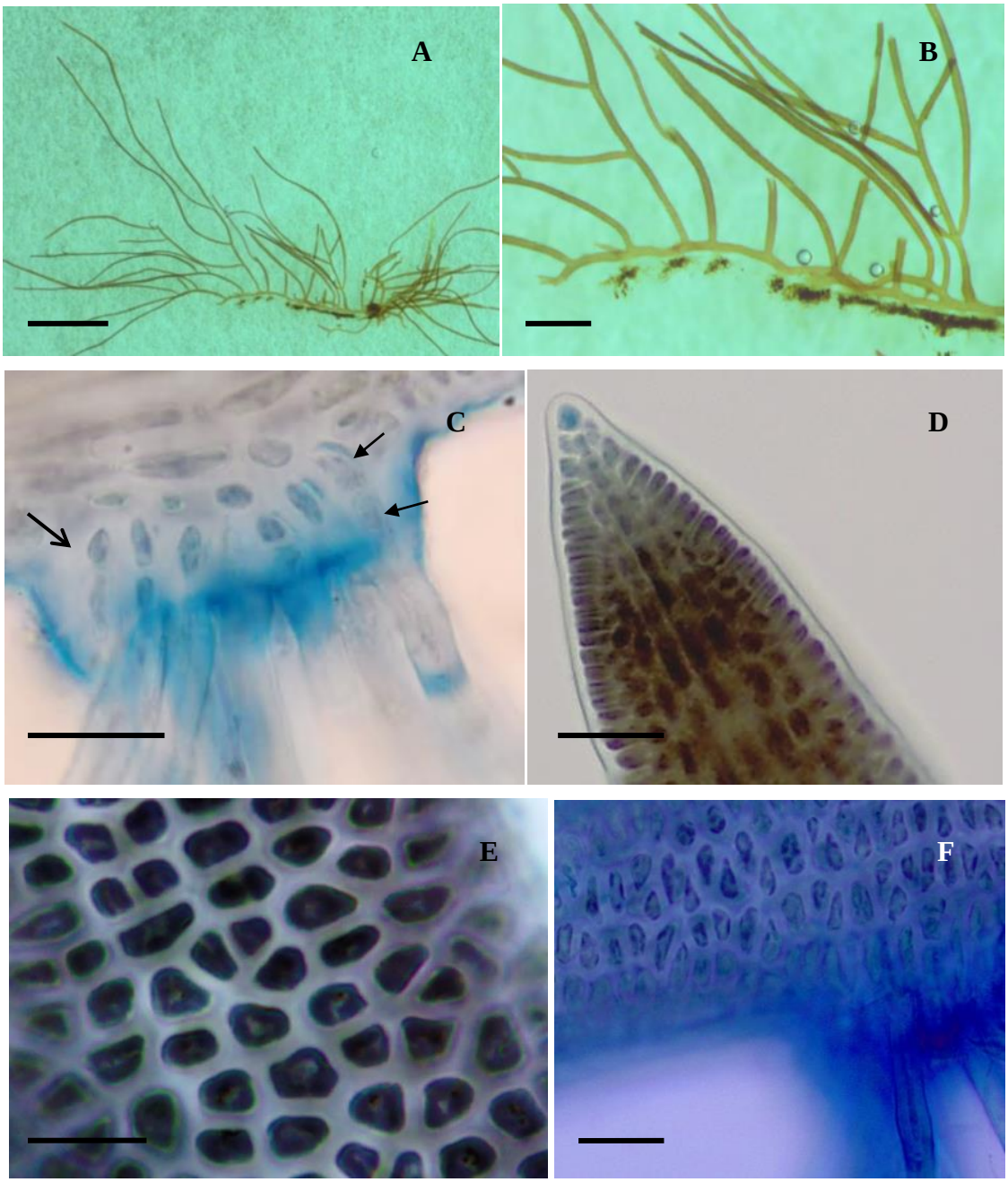

Figure 4.11: *Parviphycus* sp.1

(A) Habit of plant. Scale bar=2mm, (**B)** Fasciculated rhizoids of the creeping stolon. Scale= 500 µm **(C)** Basal cells (arrows) in the base of fasciculated rhizoids. Scale bar= 20µm, **(D)** Apical cells and distichous patern of subapical cells. Scale bar= 50µm, **(E)** Quaderate to angular cortical cells in irregular arrangement in surface view of erect branches. Scale bar=10 µm, **(F)** Transverse arrangement of long ovoid cells in surface view of stolon Scale bar=20 µm.

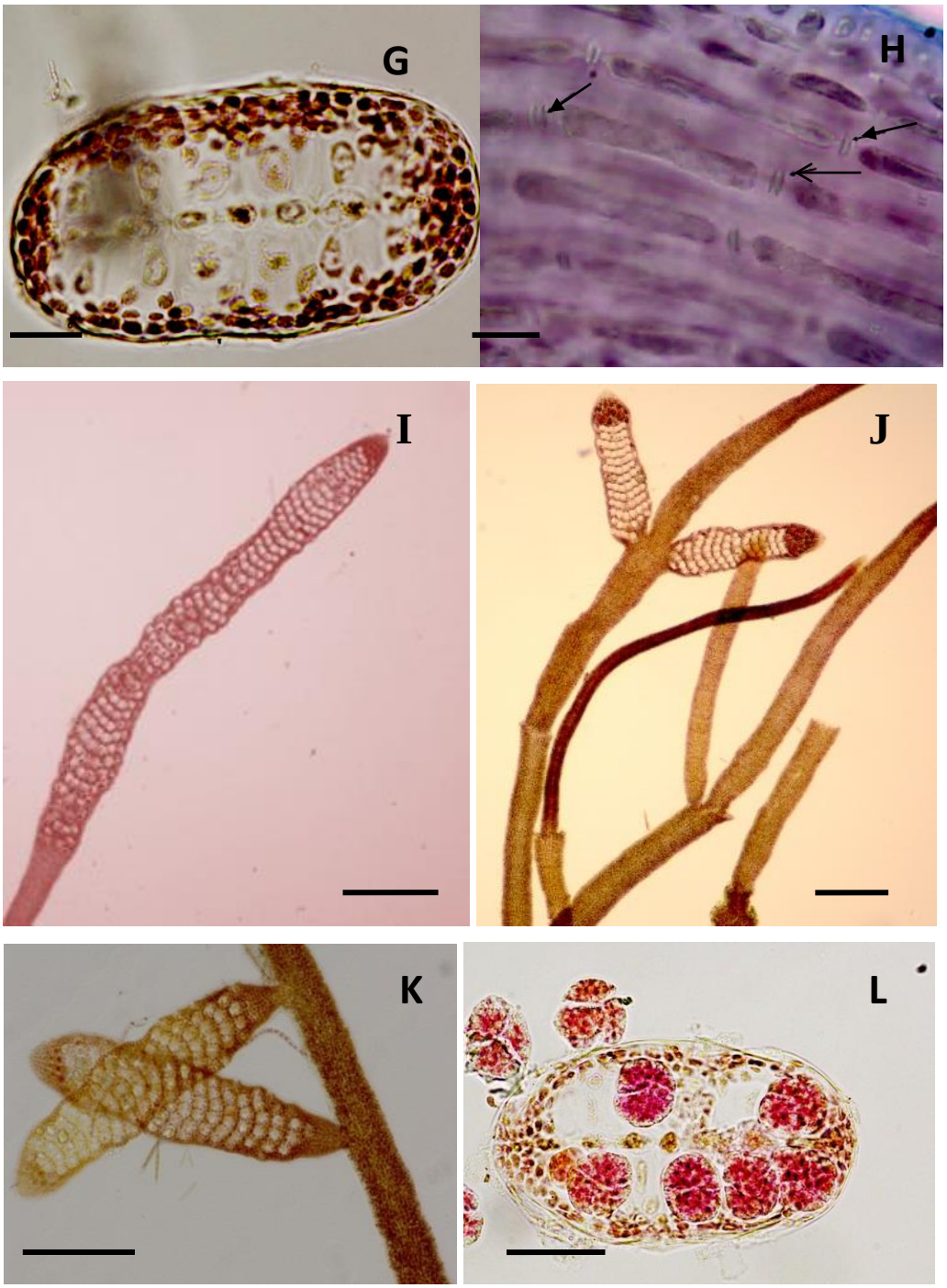

Figure 4.11: *Parviphycus* sp.1

(G) Longitudinal arrangement of long medullary cells in erect axes with clear pit connections. Scale bar=10 µm, (H) Transverse section of erect axes shows small cortical cells preclinal arrangement and axial and preaxial transverse rows of medullary cells. Scale bar=20 µm, (I) Terminal position of long tetrasporangial stichidia of tetrasporangia. Scale bar=100 µm, (J) Distichous position of tetrasporangial stichidia and acropetal development of tetrasporangia. Scale bar=200, (K) Unilateral position of tetrasporangial stichidia and acropetal development of tetrasporangia. Scale bar=200 µm, µm, (L) Transverse section of tetrasporangial stichidia, shows position of tetrasporangia in preaxial medullary cells Scale bar=40 µm.

4.1.3.3 *Parviphycus* sp.2 (Figures 4.12).

Description: Plant in a turf, blackish to brownish in color, up to 3 cm, composed of indeterminate creeping axes and extensively entangled erect branches with abundant branching in irregular pattern (Figs. 4.12A & 4.12B). Axes and branches terminated in acute to obtuse apices with a dome-shaped apical cells and distichous division of subapical cells (Fig. 4.12C); attached to substratum with aggregated groups of unicellular rhizoids (Fig. 4.12D). Axes semicompressed, 78-88 µm in width and 43-50 µm in thick, in surface view cortical cells ovoid to angular, 4.4-15.8 x 3.0-6.5 µm, mostly aligned transversely (Fig. 4.12E). In cross section of axes, two clear layers of elliptical cortical cells, with same size in two layers, 4.3-9.3 x 3.3-8.1 µm, all arranged preclinally and surrounding a medulla that contains one axial transverse row of rounded cells, 6.0-9.0 µm in diameter and two lateral preaxial smaller rounded cells (Fig. 4.12F). In some branches the arrangement of cells changed to central and pericentral and two cells in concave shape in two sides (Fig. 4.12G).

Tetrasporangial stichida mostly were in intercalary positions, in oval to conical swollen forms. Tetrasporangial stichidia were branched in polytrichous or stellate forms or, if unbranched were located in moniliform arrangement, 500 x 200 µm in size (Figs. 4.12H – 4.12I). Tetrasporangia disposed in regular rows with 4-6 sporangia in each row and upto 22 µm in diameter.

Ecology: The specimens of species were collected from the surface of the thick plastic ropes mixed with population of *Pterocladiella bartletti.*

Local distribution in Malaysia: Teluk Kemang, (2º 24 ' 54" N; 101º 51’20" E) Port Dickson, 27 Apr. 2011, J. Sohrabipoor & R. Rabiei, PSM12563.

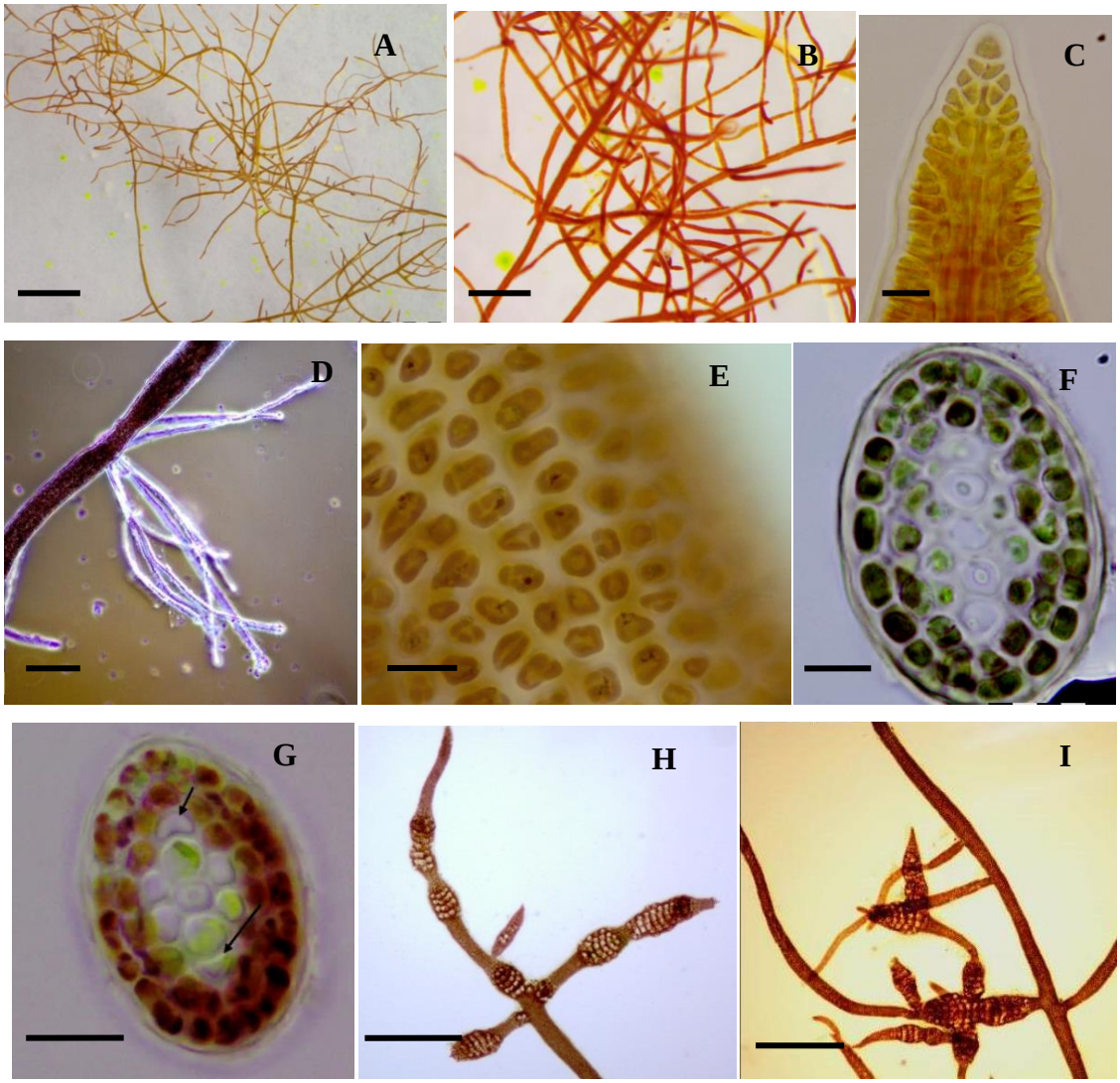

Figure 4.12: *Parviphycus* sp.2

(A) Habit of plant with long, entangled and abundantly branched axes. Scale bar=2mm, **(B)** Abundantly irregular to polytrichous branching of axes. Scale bar=1mm, **(C)** Apical cells and distichous patern of subapical cells. Scale bar= 10µm, **(D)** Fasciculated rhizoids of the creeping stolon. Scale= 100 µm, **(E)** Quadrate to oval cortical cells in transverse arrangement in surface view of erect branches. Scale bar=10 µm, **(F)** Transverse section of erect axes with 2 preclinal layers of cortical, axial and preaxial medullary cells in transverse rows. Scale bar=20 µm,**(G)** Transverse section of erect axes with 2 preclinal layers of cortical, central and pericentral arrangement of medullary cells and concave-shaped cells, (arrow). Scale bar=20 µm, **(H)** Monoliform tetrasporangial stichidia. Scale bar=500 µm, **(I)** Intercalary position of tetrasporangial stichidia. Scale bar=400 µm, (I) Polytrichous or stellate branching of intercalary tetrasporangial stichidia, Scale bar=400 µm.

4.1.3.4 *Parviphycus* sp.3 (Figure. 4.13)

Description: Plants tufted, dark brown to reddish in colour, up to 2 cm tall, composed of prostrate and erect axes, attached to the substratum by fasciculated unicellular rhizoids (Figs 4.13A). Erect axis arised from the prostrate stolon, unilaterally to polytrichously branched, mainly in upper parts of erect axis (Fig. 4.13B), semicompressed to cylindrical, 60 – 160 µm in width and 83 µm in thick. Cortical cells on erect axes longitudinally aligned; transverse section of the axes showed 2-3 layers of small elliptical cortical cells, 5.1-7.2 x 3.2-4.8 µm, preclinally arranged and medulla composed of one axial row of elliptical cells, 16-17.6 x10.2- 13.0 µm and two periaxial rows of larger round to oval cells, 20.0-25.0 x 15.0-20 µm (Fig. 4.13C).

Tetrasporangial stichidia in oval to lonceolate form, 230-900 x 120-300 µm, up to 90 µm in thick, borne apically, laterally or oppoistely on the ultimate branches of erect axes (Figs. 4.13D-G). Tetrasporangia disposed in regular transverse rows, 4-6 per row; in transverse section, tetrasporangia produced in large periaxial medullary cells (Fig. 4.13H), in rounded to oval form, up to 36 µm in diameter.

Ecology: Plants grow on the protected parts of the rocks in the high wave exposed zones and make brownish to dark purple patches on the vertical position of the rocks.

Local distributio: Pantai Bukit Kelang (5 ° 48' 5.84" N; 102 ° 36' 17" E), Kuala Terengganu, 17 Feb. 2012, J. Sohrabipoor, PSM12639.

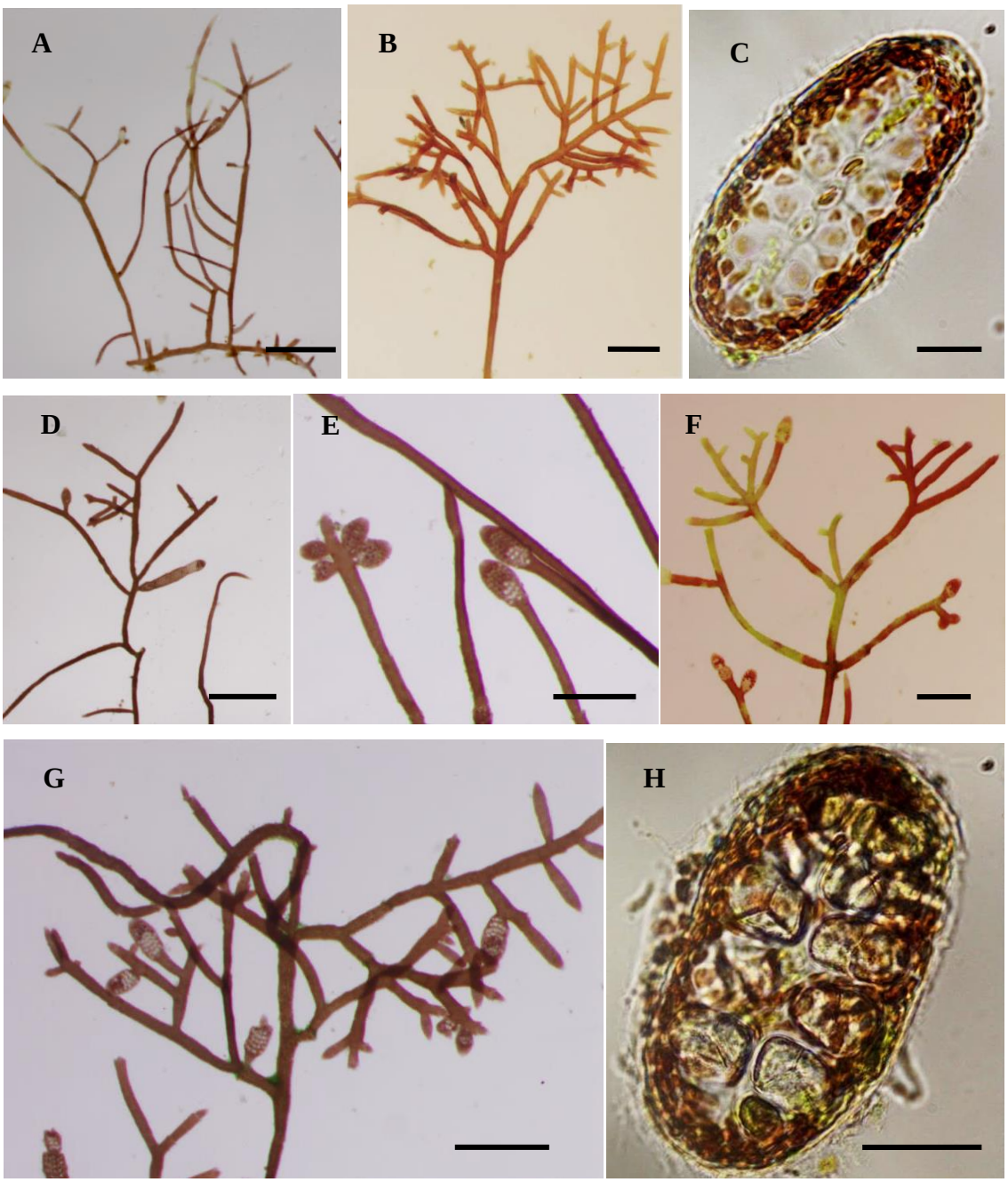

Figure 4.13: *Parviphycus* sp.3

(A) Habit of plant with unilateral branching of erect axes. Scale bar=1 mm, **(B)** Abundant branching in upper parts of erect axes. Scale bar= 500µm, (C) Transverse section of erect axes shows 2-3 layers of preclinall cortical, axial and preaxial medullary cells in transverse rows. Scale bar = 40 µm, **(D)** Lanceolate form of tetrasporangial stichidia. Scale bar=1mm, (E) Apical and opposit position of tetrasporangial stichidia. Scale bar=400 µm, **(F)** Polytrichous branching and apical tetrasporangial stichidia. Scale bar=500µm, **(G)** irregular branching and lateral tetrasporangial stichidia. Scale bar=500µm **(H** Transverse section of tetrasporangial stichidia shows the tetrasporangia within preaxial medullary cells. Scale bar= 40 µm.

Table 4.4: Morphological comparison of *Parvipphycus* species

Species / characters	*G. myriocladia*[2]	*Parviphycus adnata* [1 & 2 & 3]	*P. trinitatensis* [4 & 5]	*P. pannosus* [5]	*P. antipai* [6 & 7]
Height	1-2 cm.	1-2.5mm	Up to 5 mm	1.5 -6mm	1-2mm
Habit	Dense tufted	turfy	ni	Entangled mat	Velvet-like mat
Color		Pale red	ni		Dark red
Stolon	Cylindrical anastomosed	Cylindrical/ 40-85 µm diam.	Cylindrical / 55-75 (100) µm diam.	80-135 µm diam.	ni
Rhizoid form	Sparse grouped unicellular rhizoid	Adnate attachment with rank of single short rhizoids/25-50 x2-3 µm	ni	Rank of non-aggregated Rhizoids/200 x10-25 µm	Unconsolidated single cell rhizoids to fasciculate
Erect axis	Cylindrical to compressed/ 180 µm	Cylindrical to compressed/60-80 µm wide, 35-40µm thick	Terete/75-90 µm	Up to 8 mm high/65-115 µm wide	Terete to compressed/ 40-90 µm in diam.
Branching	Distichous, alternate, opposite to unilatera	Mostly simple or laterally branched in 1 order	Sparingly and irregularly	Mostly unbranched too irregular branching	Simple or 1-2 order branched
Cortical cells in surface view		Transversely elongated rectangular or irregular cells in prodtrat/5-15 x3-5 µm	ni	4-10 µm diam.	Transverse in prostrate axis / longitudinal in erect axis
Cortex layers	3 layers	1-2 layer	ni	2 layers	3 layers
Outermost cortical cells	Rounded in surface view (6 µm diam.)/ anticlinal in cross section(6x5 µm)	Rounded, 4-6 µm diam.	ni	Tangential elongated/ 7-10 x 3-4 µm	Irregular quadrangular / 8-9 µm in diam
Inner cortical cells		Longitudinally elongated, 10-15x4-7 µm	ni	rounded	Similar to outermost cells
Medullary cells	Rounded in cross section(7.5 µm diam.)	3-5 rows of longitudinally elongated cylindrical cells/40-60 x 7-10 µm	ni	Composed of 3-5 layers of large cells, 6-12 µm diam.	35 µm long
Tetrasporangial stichidia	Apical and lateral	shortly Pedicelate /lonceolate to cylindrical / 350-400 x 80-110 µm/ tetrasporangia 30 x20 µm	compressed to flattened, in apices, pinnately to supalmately branched/325-715 µm x98-162 µm	Flattened / apical/ one stichidium per each branch/ 250-500 x 80-180 µm	Terminal cylindrical/70 µm diam.
Tetrasporangia arrangement/size	Irregular/Tetrahedrally division / 6 per row/40x 30 µm	6 sporangia per each transvers row/20-30 µm in diam.	Cheveron-shaped/ 22-25 µm diam.	In regular rows, 6 per row/ Up to 27.5 µm	In regular transvers rows,four per each row/ni
Spermatangial stichidia		ni	ni	ni	ni
Type locality	Bombay, India	Vietnam	Trinidad, West Indies	Biarritz, France	Mexico

1.Dawson, 1954; 2. Santelices, 1977; 3. Santelices, 2002; 4. Wynne, 2011; 5. Rico *et al.*, 2002; 6. Dawson, 1953; 7. Millar & Freshwater, 2005

Table 4.4: Morphological comparison of *Parvipphycus* species (continue)

Species characters	***Parviphycus sp.1***	***Parviphycus sp. 2***	***Parviphycus sp.3***
Height	1 cm	3 cm	1-2 cm
Habit	turfy	Highly entangled	turfy
Color	reddish	Dark brown	Dark brown to reddish
Stolon	Cylindrical	indeterminate	Cylindrical to semicompress/ 119 µm
Rhizoid form	1-2 cells layer pedicelate fascicule of 2-3 cellular rhizoids, 274-388 x6.7-8.2 µm	Fasciculate rhizoids	Fasciculate rhizoids
Erect axis	semicompressed , 55-122 µm wide, 70-81 µm thick	Semicompressed/78-88 µm wide, 43-50 µm thick	60-160 µm diam/83 µm thick
Branching	Rare, one order of lateral branches	Abundantly irregular to polythrichously branched	Unilaterally to polythrichouslly
Cortical cells in surface view	Transversely to irregularly aligned of quadrate to oval cells, 4.6-8.8 x2.3-6.4 in erect axis/ Transversely elongated oval cells, 7.5-13.1 x 3-6.5 µm in creeping axis	Transversely to irregular aligned of oval to irregular shape of cortical cells, 4.4-15.8 x 3.0-6.5 µm	Longitudinally aligned
Cortical cell layers	2 layers	2 layer	2 – 3 layers
Outermost cortical cells	Priclinally elliptical to rounded small cells	Preclinally elliptical to rounded cells, 4.2-9.3 x 3.3 -8.1 µm	Preclinally elliptical /3.2-4.8 x 5.1-7.2 µm
Inner cortical cells	Preclinally elliptical as same size as outer cells	As same size of outer cells	As same size of outer cells
Medullary cells	Up to 6 rows of longitudinally medullary cells, up to 52 µm / 1 row of central and two lateral pricentral rows of large cells in treansection	One row of rounded axillary, 6-9 µm diam. and two lateral rows of smaller cells, sometimes are in circular arrangement	One row of elliptical axillary, 16-17.5 x 10-13 µm and two lateral rows of preaxial cells 20-25 x 15-20 µm
Tetrasporangial stichidia	Apical, unilateral or opposite/ up to 583 x 120 µm	Mostly intercalary, ovoid to conical in monoliform to stellate groups, 500 x 200 µm	Apical, oval to laceolate, up tp 900 x 300 µm
Tetrasporangia arrangment	Transverse open V-shaped rows/ 6-8 sporangia per row	Transverse rows, 4-6 sporangia per row	Transverse rows, 4-6 sporangia per row
Tetrasporangia diameter	15.5-30 µm	Up to 22 µm	Up tp 36 µm
locality	Telok Kemang Malaysia	Telok Kemang , Malaysia	Terengganu, Malaysia

4.2 MOLECULAR STUDIES

4.2.1 DNA EXTRACTION

Results of DNA extraction from the several specimens of the Gelidiales collected from coastlines of Malaysia using DNeasy Plant Mini Kit showed quantity and quality of the newly collected specimens were better than the stored specimens. Generally $OD_{260nm/280nm}$ ratios for most of specimens were 1.60-1.98 and concentration of the extracted DNA varied 1.45-33.35 ($\mu g.ML^{-1}$). Figure 4.14 and 4.15 as representative samples show the result of gel electrophoresis of PCR products for set primers and result of PCR purified products for *cox*I gene in gel electrophoresis.

Several DNA extractions and PCR amplification resulted in 98 partial sequences of three genes, *rbc*L, *cox*I and LSU, obtained from the Malaysian specimens. Length and nucleotides composition of these sequences has presented in Tables 4.5 and 4.6. Based on the results presented in these tables, length of sequences varied from 1232 to 1324 base pairs for *rbc*L, 600 to 1438 bp for *cox*I sequences and 868 to 908 bp for LSU sequences (Table 4.5). Nucleotide composition of these species indicated A+T:G+C ratios were about 6:4 for *rbc*L, 6.5:3.5 for *cox*1 and 5:5 for LSU (Table 4.6).

Through the multialignment of these sequences which separately analyzed in three family Pterocladiaceae, Gelidiaceae and Gelidiellaceae, some parts of the 5' and 3' ends of the sequences were trimmed because of the shorter length of the sequences acquired from GenBank.

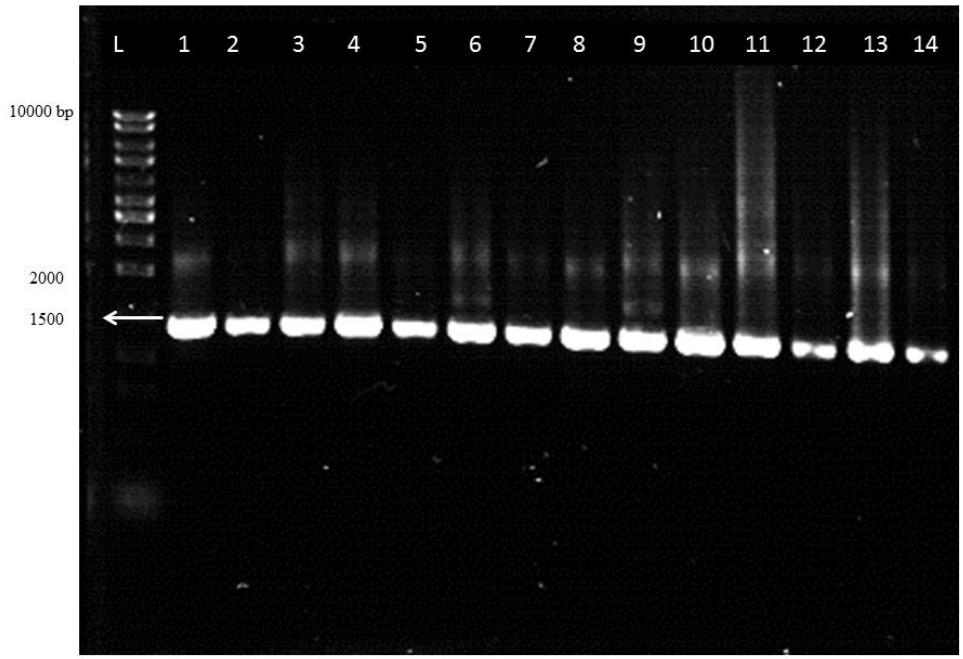

Figure 4.14: Image of PCR products for *cox*I gene from some specimens of Gelidiales on agarose gel (1%). (L: 1Kb plus DNA ladder and 1 to 14 selected specimens of Malaysian Gelidiales)

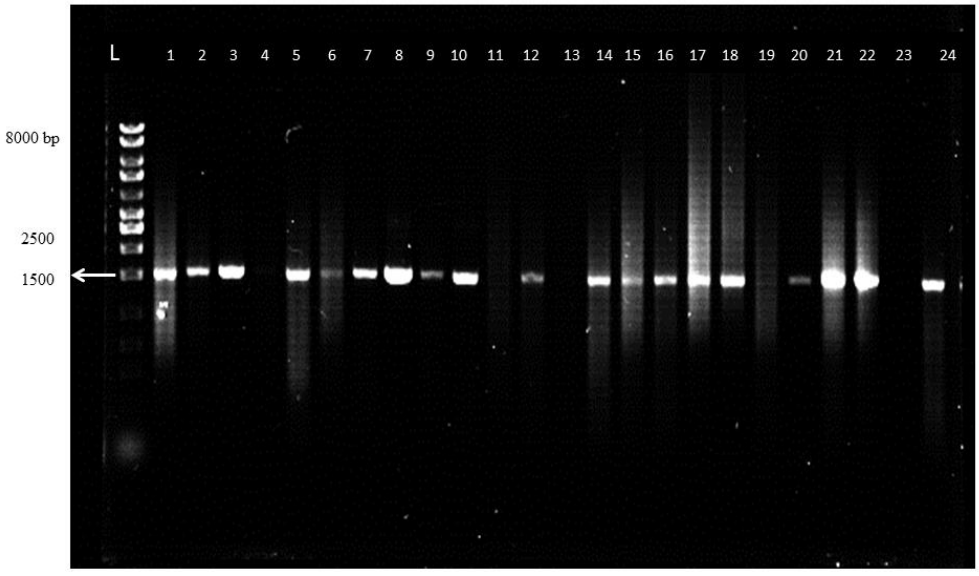

Figure 4.15: Image of purified PCR products of cox1 gene from some specimens of Malaysian Gelidiales on agarose gel 1% (L: 1Kb plus DNA ladder and 1 to 244 selected specimens of Malaysian Gelidiales)

Results of phylogenetic analyses of the species were explained after their morphological features, that were classified in three families of Gelidiales. Because of high number of sequences generated in this study combined with the acquired sequences from GenBank their phylogenetic analyses were conducted separately in three family Pterocladiaceae, Gelidiaceae and Gelidiellaceae. Therefore results are presented in three parts. Finally for finding the phylogenetic relationships of all Gelidiales, three phylogenetic trees were constructed to clarify the unambiguous taxonomic position of some taxa such as *Aphanta* and *Gelidium* sp.nov.2 from Malaysia as well as *Pterocladia lucida, Ptilophora* and *Capreolia*.

Table 4.5: The length of *rbc*L, *cox*I and LSU genes sequences.

Species	**Length in Base Pair**		
	***rbc*L**	***cox*1**	**LSU**
Aphanta sp.	1556	810-1438	868-896
Pterocladiella caerulescens	1226-1290	730-1408	905
P. beachiae	1250-1290	778-1308	906
Pterocladiella sp. nov.1	1280-1290	1308	
Pterocladiella sp. nov.2	1290	1308	875-904
P. bartlettii	1288-1290	769-1308	880-908
Gelidium sp. nov.1	1324	1375-1393	
G. perpusillum	1230 -1324	1393	
Gelidium sp.nov.2	1253-1320	1374-1393	
Gelidiella acerosa	1232-1294	1204- 1365	
Parviphycus spp.	1232-1294	600-1365	

Table 4.6: Nucleotide composition of three genes, *rbc*L, *cox*I and LSU obtained from the Gelidiales species identified from Malaysian coastlines

Species	***rbc*L**				***cox*1**				**LSU**			
	A (%)	**C (%)**	**G (%)**	**T (%)**	**A (%)**	**C (%)**	**G (%)**	**T (%)**	**A (%)**	**C (%)**	**G (%)**	**T (%)**
Gelidium sp.nov.1	30.93	16.30	21.23	31.54	26.49	15.29	18.32	39.70				
G. perpusillum	31.18	17.13	21.27	30.41	26.27	15.08	18.59	40.06				
Gelidium sp.nov.2	32.14	16.11	20.90	30.85	25.26	16.16	19.72	38.86				
Aphanta sp.	31.04	17.03	21.08	30.85	25.53	15.99	19.21	39.28	25.31	20.85	30.21	23.63
Pterocladiella beachiae	31.01	17.67	21.32	30.00	25.16	14.56	19.67	40.61	25.75	19.89	29.95	25.41
P. caerulescens	31.05	17.44	21.60	29.91	26.24	14.94	18.92	39.90	25.72	19.76	29.03	25.50
Pterocladiella sp.nov.1	30.39	17.29	21.55	30.79	26.96	15.22	18.42	39.40				
Pterocladiella sp.nov.2	31.71	16.98	21.01	30.31	26.74	14.94	18.28	40.04	25.6	20.5	30.18	24.3
P. bartlettii	30.85	17.75	21.43	30.20	27.09	14.87	18.29	39.76	25.2	20.16	30.19	25.6
Gelidiella acerosa	30.76	17.47	21.10	30.68	28.06	15.97	17.97	38.02				
Parviphycus spp.	30.93	16.96	21.59	30.52	28.45	15.91	18.04	37.61				

4.2.2 PHYLOGENETIC ANALYSES OF FAMILY PTEROCLADIACEAE

The alignment of 52 partial *rbc*L gene sequences of Pterocladiaceae including 24 sequences of Malaysian specimens, 39 partial *cox*I gene sequences including 22 sequences of Malaysian specimens and 27 partial LSU gene sequences including nine sequences of Malaysian specimens were straightforward, necessitating neither the introduction of gaps nor ambiguous alignments. LSU gaps were introduced as missing nucleotides. Lists of sequences acquired from GenBank are listed in Appendix 17.

Parameters for phylogenetic analyses of partial *rbc*L, *cox*I and LSU genes sequences of Pterocladiaceae including Malaysian specimens using ML and MP methods are summarized in Table 4.7. The *rbc*L alignment was 1295 bp in length, the *cox*I alignment length was 603 bp and LSU was 1124 bp (Table 4.7). Separate phylogenetic analyses within Pterocladiaceae using ML, MP and BI methods resulted in phylogenetic trees that showed similar but not fully congruent topologies; therefore only results from the ML analyses of each genetic marker are presented. Bootstrap values and percentage of posterior probabilities resulting from the three methods of analyses are indicated on the branches of the ML phylogeny (Figs. 4.16 – 4.18). Corrected mean distance based on HKY85 model for three set sequences of *rbc*L, *cox*I and LSU genes are presented in Tables 4.8- 4.10.

Table 4.7: Nucleotide composition and statistics for maximum parsimony and maximum likelihood analyses of the three set sequences of *rbc*L, *cox*I and LSU genes in phylogenetic analyses of Pterocladiaceae.

	***rbc*L**	***cox*I**	**LSU**
Number of taxa	52	39	27
Nucleotides (base pair)	1295	603	1124
Base frequency (A/C/G)	0.320/0.151/0.199	0.332/0.116/0.124	0.252/0.206/0.294
Variable sites (%)	407 (31.4)	219 (36.3)	151(13.4)
I (invariable site rate)	888(68.6)	384(63.7)	973(86.6)
Informative sites (%)	359 (27.7)	188 (31.2)	114(10.1)
Selected model	GTR + G +I	JI+G+I	TVM+G
α (shape parameter)	0.2173	0.1200	0.1002
Ln Likelihood	-5681.834	-3090.234	-2923.101

Phylogenetic analyses of 24 new *rbc*L sequences obtained from Malaysian species of family Pterocladiaceae with 26 sequences of Pterocladiaceae from GenBank and two sequences of *Gelidiella acerosa* (EU146836) and *Gelidium japonicum* (HM629830) as outgroups, as well as analyses of 22 *cox*I gene sequences from Malaysian *Pterocladiella* specimens with 17 sequences from GenBank sequences containing two sequences of *Gelidiella acerosa* (HM102421) and *Gelidium divaricatum* (HM629865) as outgroups, and nine LSU sequences of Malaysian specimens with 15 sequences of GeneBank and three sequences of *Ptilophora coppejansii* (AF512184), *Gelidium japonicum* (AF512185) and *Capreolia implexa* (AF039545) as outgroups, led to the determination and identification of six species of *Pterocladiella* from Malaysian coastlines.

The ML trees and all analyses inferred from *rbc*L (Fig. 4.16), *cox*I (Fig. 4.17) and LSU (Fig. 4.18) genes resolved sequences of Malaysian specimens into six species: *P. bartlettii*, *P. beachiae* and *P. caerulescens* and three taxa which were not identified by

any known species of Pterocladiceae, and appear to be new species, which including *Pterocladiella* sp. nov. 1, *Pterocladiella* sp. nov.2 and *Aphanta* sp. The two species, *P. bartlettii* and *P. beachiae*, are new records for Malaysia.

Three sequences of *Pterocladiella* sp. nov.1 specimens from Port Dickson, Malaysia, had identical *rbc*L sequences and their *cox*I sequences differed only by 0-0.2% (Fig. 4.16, Table 4.8). *Pterocladiella* sp. nov.1 had a sister relationship with a clade composed of *P. caerulescens* from Malaysia and Hawaii, *P. beachiae* from Malaysia and Central America, *P. psammophila and P. australafricanensis* from South Africa (Fig. 4.16).

Five specimens of the new species *Pterocladiella* sp. nov.2 from Teluk Kemang in west coast and Pulau Pinang in northwest coast of Peninsular Malaysia had very similar *rbc*L and *cox*I sequences (pairwise divergences of 0–0.3% for *rbc*L and 0–0.2% for *cox*I) (Figs 4.16 & 4.17 and Tables 4.8 & 4.9). In *rbc*L gene analyses, *Pterocladiella sp*. nov.2 supported with high to full support bootstrap values (ML=99%, MP=100%, BI=100%) and showed a sister relationship with *P. bartlettii* from Costa Rica, Texas and Malaysia, and this clade formed a monophyletic group with *P. melanoidea* (Schousboe ex Bornet) Santelices & Hommersand from Spain, in which *P. melanoidea* was basal (Fig. 4.16). In LSU gene sequences analyses, one sequence of the newly proposed species, *Pterocladiella* sp. nov.2, showed a sister relationship with *P. melanoidea* and grouped in a monophyletic clade with *P. bartlettii* (Fig. 4.18). The interspecific divergence of *Pterocladiella* sp. nov.2 from *P. bartlettii* (8.63-9.01% for *rbc*L, 10.9-11.6% for *cox*I and 0.9% for LSU) (Tables 4.8-4.18) and from *P. melanoidea* (11.2-11.3% in *rbc*L and 0.9% in LSU) (Tables 4.9 & 4.10) clearly resolved *Pterocladiella* sp. nov.2 as a distinct species. This species also showed high divergence

from *Pterocladiella* sp. nov.1 (12-12.3% for *rbc*L and 17.4-17.6% for *cox*I) (Tables 4.8, 4.9), although both grow in the same locations, Teluk Kemang.

Two *rbc*L sequences, five *cox*I sequences and three LSU sequences of Malaysian *Aphanta* specimens collected from Port Dickson and Kota Kinabulu, Sabah, eastern Malaysia, in all analyses for three genes formed distinct identical clade with strong to full bootstrap support (Figs. 4.16- 4.18). In *rbc*L analyses these sequences were grouped with *Aphanta pachyrrhiza* Tronchin & Freshwater from South Africa by strong to full support (ML=100%, MP=100% & BI=99%) (Fig. 4.16). Phylogenetic analyses of *cox*I gene showed the sequences of these specimens created a distinct new clade with strong to full support (ML=98%, MP=100% & BI=100%), out of the all clades in Pterocladiaceae (Fig. 4.17).

In LSU sequences analyses, sequences of the *Aphanta* specimens grouped in a same clade with full bootstrap support in all three analyses methods for LSU gene. This identical clade showed sister relationship with the only one LSU sequence of *Aphanta pachyrrhiza* from South Africa (Fig. 4.18).

Pairwise sequences divergence of these three genes in Malaysian specimens of *Aphanta* was low (0.0% in *rbc*L, 0.0-0.3% in *cox*1 & 0.0-0.1% in LSU) but pairwise divergences of the Malaysian sequences from South African species, *A. pachyrrhiza* (9.8-9.9% for *rbc*L & 2.2-2.3% for LSU) were the ranges represent two distinct species (Tables 4.8 - 4.10). Phylogenetic analyses of the three genes sequences showed *Aphanta pachyrrhiza* from South Africa and *Aphanta* sp. from Malaysia formed a paraphyletic clade in the Pterocladiaceae.

Another interesting observation is that *Pterocladia lucida* from Australia and New Zealand were grouped as an independent monophyletic clade from other family members of *Pterocladiella* in LSU analyses but in *rbc*L analyses this species showed as basal group in the family of Pterocladiaceae but was weakly supported with boostrap value of 68% for MP (Figs. 4.16 & 4.18). Pairwise sequences divergence of the species from other members of Pterocladiaceae (> 13% in *rbc*L and > 4.6% in LSU) showed this species is distant from family Pterocladiaceae (Tables 4.8 & 4.10).

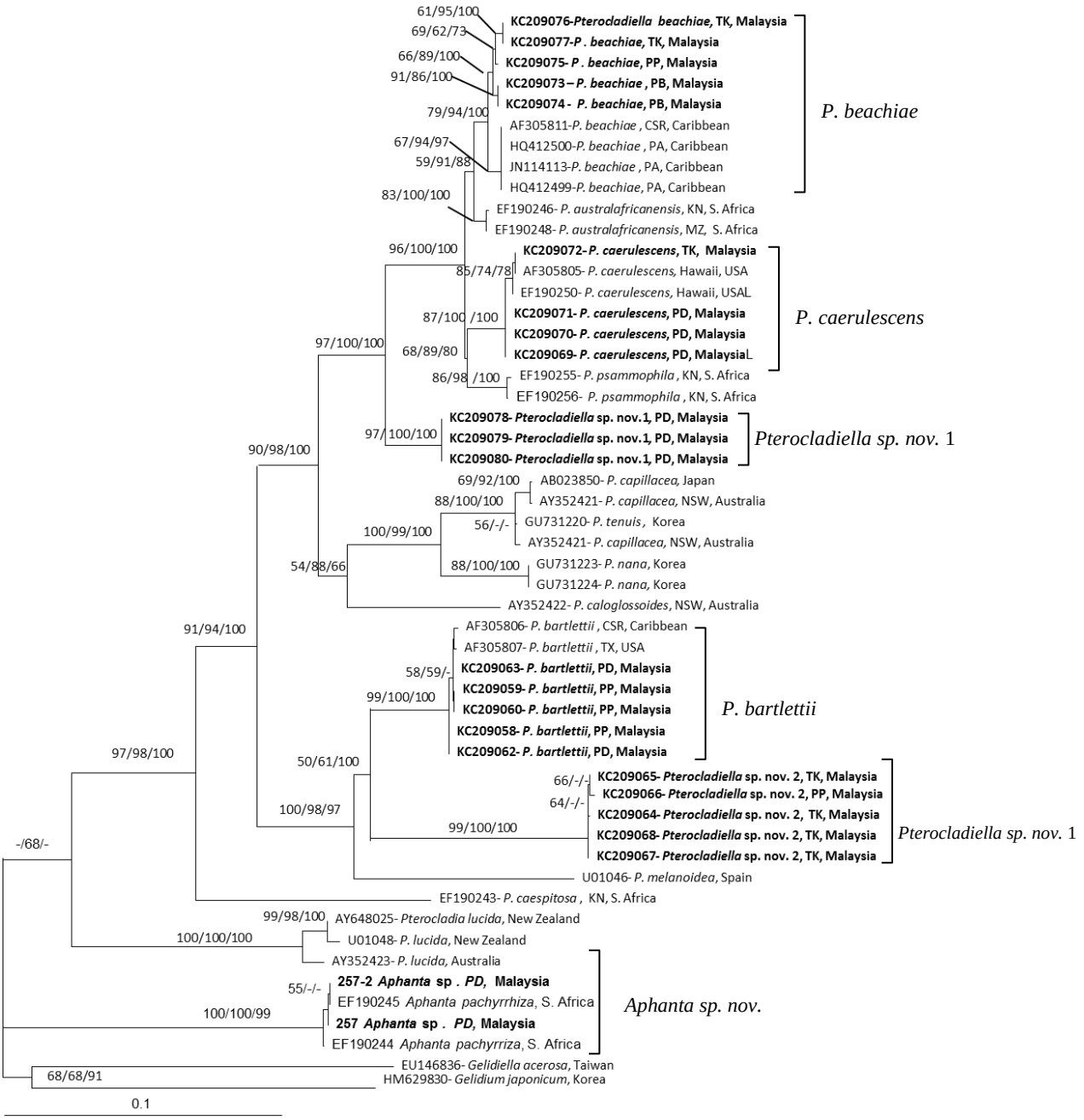

Figure 4.16: Maximum likelihood tree based on 52 partial *rbc*L sequences of Pterocladiaceae including 24 sequences of Malaysian specimens. ML and MP bootstrap value and percentage of posterior probabilities for BI are appended above the internodes respectively. The taxa in bold are the sequences of specimens from Malaysia. (CSR = Costa Rica, KN =KwaZulu-Natal, MZ = Mozambique, PA= Panama, PB =Palau Bear, PD= Port Dickson, PP= Palau Pinang, TK =Teluk Kemang, TX =Texas, USA=United States of America).

In the *rbc*L analyses (Fig. 4.16), nine sequences from Malaysian specimens which morphologically were similar to *P. caerulescens* were grouped in a complex clade comprising *P. caerulescens, P. beachiae* Freshwater, *P. psammophila* Tronchin & Freshwater and *P. australafricanensis* Tronchin & Freshwater. Five *P. beachiae* specimens from Pulau Besar, Pulau Pinang and Port Dickson were moderate to robustly grouped with *P. beachiae* from Caribbean Costa Rica, and Panama (ML=79%, MP=94%, BI=100%). The intraspecific pairwise divergence of *rbc*L sequences between *P. beachiae* from Malaysia and *P. beachiae* from the Caribbean and Central America was very low (0.6–0.8% for *rbc*L). Four samples of *P. caerulescens* from Teluk Kemang and Port Dickson formed a subclade with *P. caerulescens* from Hawaii with strong to full support (ML=87%, MP=100%, BI=100%) (Fig. 4.16) and low pairwise divergence (0.4%) (Table 4.8).

In the *cox*I ML tree, three sequences of *P. caerulescens* from Port Dickson were also moderate to robustly grouped with the Hawaiian sequences of *P. caerulescens* (ML = 97%, MP = 87%, and BI =99%) (Fig. 4.17) and there was low sequence divergence among Malaysian specimens (0-0.3%) and among all sequences of *P. caerulescens* (0.8-1.3%) (Table 4.9). Three sequences of *cox*I gene from Malaysian specimens of *P. beachiae* were grouped together with *P. beachiae* from Costa Rica and Panama (Fig. 4.17) with moderate to strong support (ML=70%, MP=84% and BI=96%).

Sequences pairwise divergences between *cox*I sequences of Malaysian and Caribbean specimens of *P. beachiae* (2.0-2.4%) were higher than the intraspecific divergence among Malaysian sequences (0.7- 0.8%) and among the Caribbean sequences (0-0.3%).

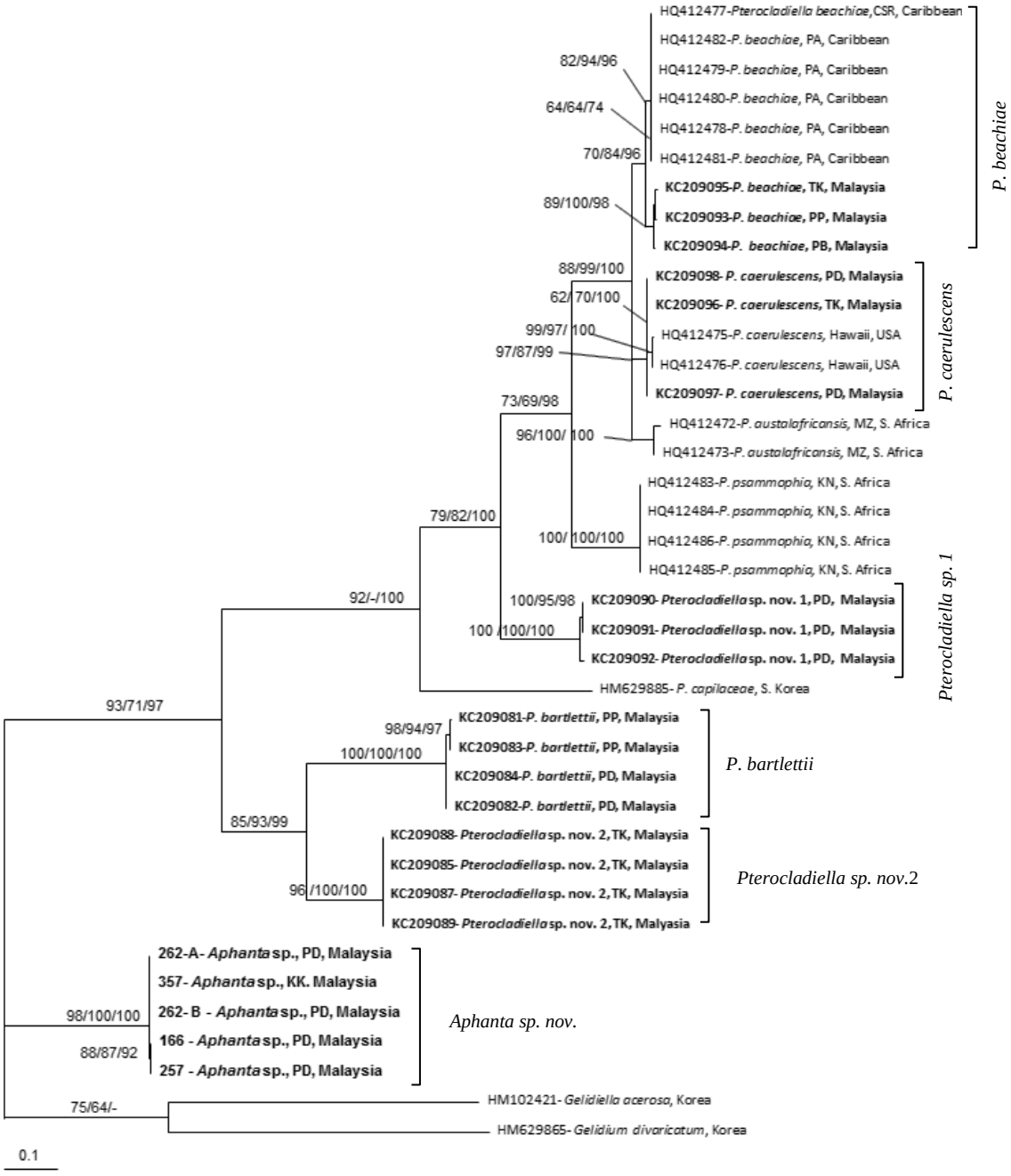

Figure 4.17:. Maximum–likelihood tree of 39 *cox*I sequences of Pterocladiaceae including 22 sequencea of Malaysian specimens. ML and MP bootstrap value and percentage of posterior probabilities for BI are appended above the internodes respectively. The taxa in bold indicate the species from Malaysia. (CSR=Costa Rica, KK=Kota Kinabalu, KN=KwaZulu-Natal, MZ=Mozambique, PA=Panama, PB=Pulau Besar, PD=Port Dickson, PP= Pulau Pinang, TK=Teluk Kemang, TX =Texas).

Interspecific divergence between *P. beachiae* and *P. caerulescens* from Malaysia (5.1-5.5%) is higher than the divergence of these two species from the Caribbean and Hawaii (4.5-4.7%) (Table 4.9), showing that *P. beachiae* is distinct from *P. caerulescens*.

In LSU analyses two sequences of P. *beachiae* and one sequence of *P. caerulescens* from Malaysian specimens all were grouped with *P. caerulescens* from Hawaii, *P. beachiae* from Costa Rica and *P. australafricanensis* from South Africa (Fig. 4.10).

Phylogenetic analyses using both *rbc*L and *cox*I genes grouped sequences of five Malaysian specimens of *P. bartlettii* from Pulau Pinang and Teluk Kemang in a monophyletic clade. In *rbc*Lanalyses they were grouped with *P. bartlettii* from Costa Rica in the Caribbean Sea and Texas in USA, and were robustly supported (ML= 99%, MP= 100% & BI= 100%) (Fig. 4.16). In *cox*I analyses this group was fully supported in three analyses methods (ML= 100%, MP= 100% & BI=100%).

This species showed a sister relationship with the proposed new species, *Pterocladiella* sp. nov.2, from Malaysia and both were grouped into a monophyletic clade with *P. melanoidea* from Spain.

*Cox*I gene sequences of *P. bartlettii* from Malaysia also formed a sister relationship with the subclade comprising the sequences of the new species, *Pterocladiella* sp. nov.2, from Malaysia (Fig. 4.17). There was very low sequence divergence among sequences of Malaysian *P. bartlettii* (0-0.2% in *rbc*L & 0-0.9% in *cox*I) and low intraspecific divergence from *P. bartlettii* from Haiti and Texas (0-0.3%) (Tables 4.8).

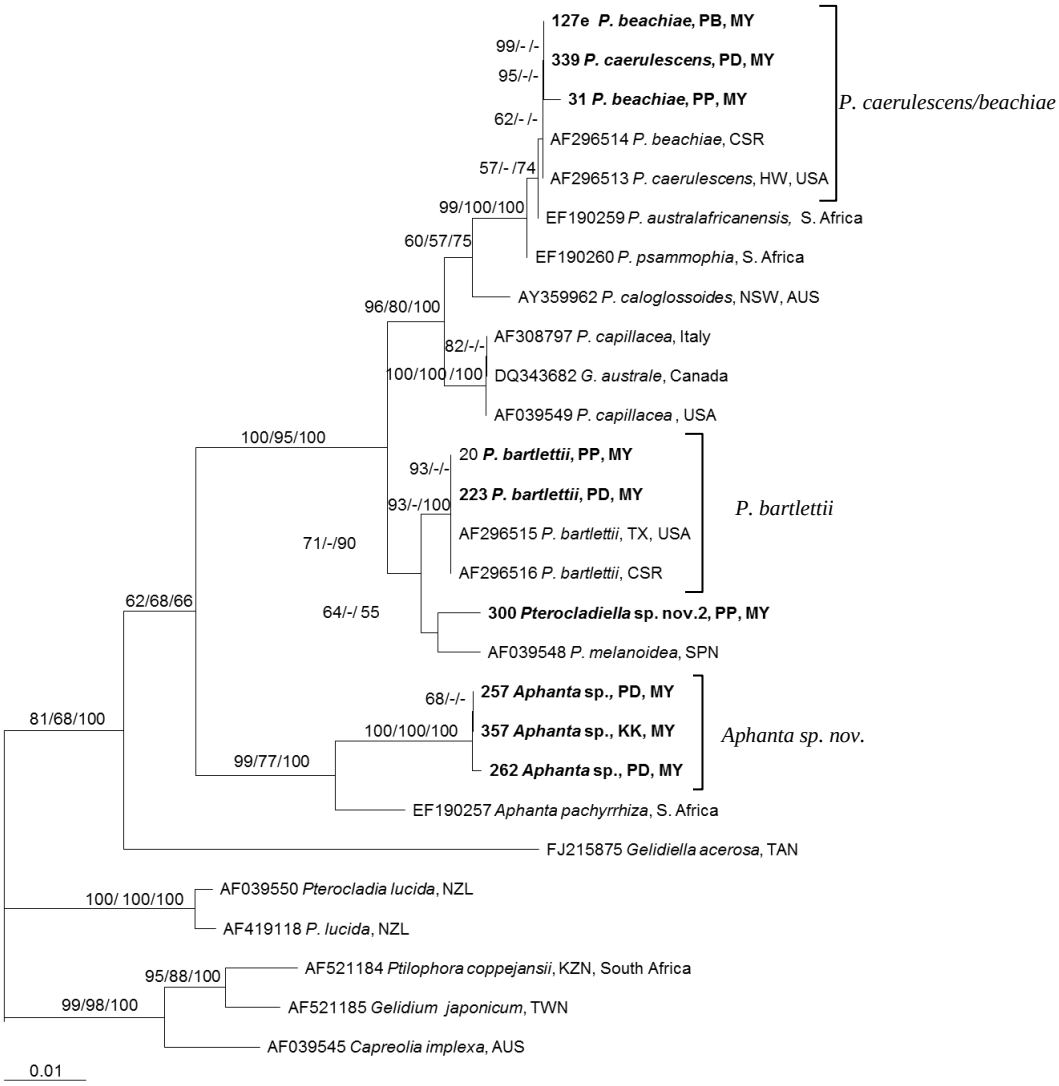

Figure 4.18: Maximum–likelihood tree of 27 LSU sequences of Pterocladiaceae including 9 sequences of Malaysian specimens. ML, MP bootstrap value and percentage of posterior probabilities for BI are appended above the internodes respectively. The taxa in bold indicate the species from Malaysia. (CSR= Costa Ricaa, HW= Hawaii, KK= Kota Kinabalu, KN =KwaZulu-Natal, MY= Malaysia, NZL= New Zealand, PB =Pulau Besar, PD= Port Dickson, PP= Pulau Pinang, SPN= Spain, TAN= Thailand, TWN= Taiwan, TK =Teluk Kemang, TX= Texas).

High pairwise divergences between *Pterocladiella* sp. nov.2 and *P. bartlettii* from Malaysia (8.6-8.7% in *rbc*L and 10.9-11.6% in *cox*I) and *P. bartlettii* from Haiti and Texas (8.8-9.0% in *rbc*L) resolved them as two distinct species. The *rbc*L divergences between *P. bartlettii* from Malaysia, Haiti and Texas and *P. melanoidea* (8.4–9.5%) likewise showed that they are two distinct species. In LSU gene sequences data two sequences of Malaysian specimens of *P. bartlettii* were grouped with *P. bartlettii* from Costa Rica and Texas by strong to full support (Fig. 4.18) with no pairwise divergence divergence (Table 4.10).

Table 4.8: Pairwise distance between multialignment sequences of partial *rbc*L region of Pterocladiaceae species from Malaysia and the sequences acquired from GenBank excluding gaps and ambiguities. Distance between taxon pairs calculated using uncorrected model. [Abbreviations for localities:AUS=Australia, CSR= Costa Rica, NZL=New Zealand, PA= Panama, SA= South Africa, HW=Hawaii, MY= Malaysia, SP=Spain, TX= Texas]

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
[1] *P. beachiae* (CSR & PA)	0.00														
[2] *P. beachiae* (MY)	0.6 -0.8	0.0 - 0.4													
[3] *P. caerulescens* (HW)	2.2	2.4-2.6	0.00												
[4] *P. caerulescens* (MY)	2.3	2.6-3.5	0.7	0.00											
[5] *P. australafricanensis*(SA)	1.3-1.4	1.7-1.6	2.1-2.1	2.3-2.4	0.1										
[6] *P. psammophila* (MY)	2.1-2.6	2.6-2.8	2.4-2.7	2.6-2.8	2.1-2.4	0.1									
[7] *P. caloglossoides*(AUS)	9.2	9.1-9.2	9.1-9.4	9.6	8.7-8.8	8.2-9.6	-								
[8] *Pterocladiella* sp.1 (MY)	5.6	5.4-5.6	5.7-5.7	5.9	5.3-5.3	5.5-6.0	9.2	0.0							
[9] *Pterocladiella* sp.2 (MY)	12.7-13.0	12.8-13.2	13.6-14.1	14.1-14.3	13.1-14.5	12.5-13-2	13.2-13.5	12.0-12.3	0.0 -0.7						
[10] *P. bartlettii* (TX & CSR)	12.0-12.1	11.5-11.8	11.9-12.1	12.2-13.3	11.9-12.1	11.5-12.2	11.0-11.1	10.7-10.7	8.6 - 9.0	0.3					
[11] *P. bartlettii (*MY)	11.2	11.4-11.6	11.8-11.9	12.1	11.8-12.1	11.7-12.2	11.0-11.2	10.7	8.6-8.7	0.2-0.3	0.0-0.2				
[12] *P. melanoidea* (SPN)	11.9	11.7-12.2	12.2-13.5	12-12.2	12.0-12.1	11.7	12.6	12.1	11.2-11.3	8.4-9.5	9.4-9.5	-			
[13] *A. pachyrrhiza* (SA)	14.9-15.0	14.9-15.1	15.3-15.7	15.7-15.8	15.1-15.3	14.9-15.1	14.5-14.6	14.5-14.6	15.3-16.6	15.2-15.5	15.2-15.3	14.5-14.6	0.0		
[14*] Aphanta sp*. (MY)	15.6	15.6-15.8	16.0-16.2	16.7	15.5-15.7	15.9-16.0	14.3	15.1	17.5	15.4-15.5	15.4-15.5	14.2	10.0	0.0	
[15] *Pterocladia lucida* (AUS & NZL)	14.2-15.1	14.4-15.2	15.0-16.1	15.7-15.8	14.8-15.9	14.4-15.6	14.3-14.7	14.0- 14.5	13.4-14.6	13.1-14.1	13.4-14.0	14.2-14.7	14.2-15.8	14.5-17.3	0.3-2.1

Table 4.9: Pairwise distance between multialignment sequences of partial *cox*I region of Pterocladiaceae species from Malaysia and the sequences acquired from GenBank excluding gaps and ambiguities. Distance between taxon pairs calculated using uncorrected model. [Abbreviations for localities: CSR= Costa Rica, PA= Panama, SA= South Africa, HW=Hawaii, MY= Malaysia].

		1	2	**3**	**4**	5	6	7	**8**	9	10
1	*P. beachiae* (CSR & PA)	0.0-0.3									
2	*P. beachiae* (My)	2.0-2.7	0.7-0.8								
3	*P. caerulescens* (HW)	4.5-4.7	4.4-4.5	0.2							
4	*P. caerulescens* (MY)	4.3-4.5	5.1-5.5	0.8-1.3	0.0-0.3						
5	*P. australafricanensis* (SA)	4.7-6.0	5.3-6.8	5.2-6.1	5.0-5.8	1.4					
6	*P. psammophila* (MY)	9.2-9.8	10.2 -10.4	10.6-11.0	10.6-10.8	11.2-12.7	0.0-0.2				
7	*Pterocladiella* sp.1 (MY)	12.3-12.8	12.3-13.2	12.9-13.3	12.5-12.8	13.1-13.9	13.4-14.1	0.0-0.2			
8	*Pterocladiella* sp.2 (MY)	16.6-17.1	18.8-18.9	17. 9-18.1	17.9-17.9	18.4-19.5	18.6-19.5	17.4-17.6	0.0-0.2		
9	*P. bartlettii* (MY)	16.2-16.9	17.7-18.5	17.6-18.4	17.9-18.4	17.4-18.9	19.6-20.3	19.7-20.1	10.9-11.6	0.0-0.9	
10	*Aphanta sp.*(MY)	15.5-16.0	16.8-17.1	18.3-18.6	18.1-18.6	17.5-18.6	19.2-19.6	19.8-19.9	17.5-18.4	17.5-18.3	0.0-0.3

Table 4.10: Pairwise distance between multialignment sequences of partial LSU region of Pterocladiaceae species and the sequences acquired from GenBank, excluding gaps and ambiguities. Distance between taxon pairs calculated using uncorrected model. [Abbreviations for localities: AUS= Australia CSR= Costa Rica, ITL=Italy, MY= Malaysia, PA= Panama, SA= South Africa, HW=Hawaii, , SPN=Spain, TA=Taiwan, TAN=Tanzania]

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
[1] *Aphanta sp. (*My)	**0.0-0.1**																
[2] *A. pachyrrhiza* (SA)	**2.2-2.3**	**-**															
[3] *P. capillacea* (ITL& USA)	**5.1-5.4**	**4.3-4.4**	**0.0**														
[4] *P. caerulescens* (HW)	**5.6-5.8**	**4.1-4.2**	**1.4**	**-**													
[5] *P. caerulescens*(My)	**5.6-5.7**	**4.8**	**1.5-1.6**	**0.0-0.1**	**-**												
[6] *P. beachiae* (HA)	**5.6-5.8**	**4.1**	**1.3-1.4**	**0.0-0.09**	**0.0**	**-**											
[7] *P. beachiae* (MY)	**5.6-5.8**	**4.8-5.0**	**1.5-1.8**	**0.0-0.3**	**0.2**	**0.0-0.2**	**0.2**										
[8] *P. bartlettii* (CSR)	**5.2-5.4**	**3.8-3.9**	**1.1-1.6**	**1.3-1.4**	**1.8-1.9**	**1.3-1.4**	**2.4**	**0.0**									
[9] *P. bartlettii* (MY)	**5.2-5.4**	**4.4**	**1.7-1.8**	**2.4-2.5**	**2.4**	**2.4**	**2.4-2.7**	**0.0**	**0.0**								
[10] *Pterocladiella* sp.2 (MY)	**5.4-5.5**	**5.8**	**1.7-1.8**	**2.6-2.8**	**2.6**	**2.6**	**2.7-2.9**	**1.1**	**1.1**	**-**							
[11] *P. melanoidea* (SPN)	**4.9-5.0**	**4.0**	**1.3-1.4**	**2.3**	**2.7**	**2.2**	**2.6-2.9**	**0.9-1.0**	**0.9**	**0.9**	**-**						
[12]*P.caloglossoides* (AUS)	**5.6-5.7**	**3.8**	**1.1-1.2**	**1.2-1.3**	**1.3**	**1.9**	**1.3-1.5**	**1.5**	**1.7**	**1.8**	**1.8**	**-**					
[13] *P. lucida* (AUS & NZL)	**6.3-6.8**	**5.0-5.2**	**4.9-5.1**	**5.0-5.1**	**5.8-5.9**	**5.0-5.1**	**5.8-6.1**	**4.4-4.5**	**5.0-5.1**	**5.3-5.8**	**5.0-5.1**	**4.6-4.9**	**-**				
[14] *G. japonicum* (TA)	**6.4-6.5**	**5.2**	**5.7-6.0**	**6.2-6.3**	**7.1**	**6.1**	**7.1-7.4**	**5.5-5.6**	**6.4**	**6.6**	**5.8**	**5.9**	**4.3**	**-**			
[15] *Ptilophora coppejansii* (SAF)	**6.5-6.6**	**5.4**	**6.3-6.4**	**6.5-6.6**	**7.3**	**6.4**	**7.3-7.5**	**5.9-6.0**	**6.6**	**7.0**	**6.1**	**6.1**	**4.4-4.5**	**2.2**	**-**		
[16] *Capreolia implexa*(AUS)	**6.3-6.4**	**5.1**	**5.9-6.1**	**5.8-5.9**	**5.8-5.9**	**5.8**	**6.8-7.1**	**5.3-5.4**	**6.3**	**6.8**	**5.5**	**5.9**	**4.0-4.2**	**2.1**	**2.2**	**-**	
[17] *Gelidiella acerosa*(TAN)	**6.8-6.9**	**5.4**	**6.7-6.8**	**6.6-6.7**	**8.1**	**6.7**	**8.1-8.3**	**6.3**	**7.7**	**7.8**	**6.2**	**7.1**	**6.7-6.9**	**5.7**	**6.6**	**5.8**	**-**

4.2.3 PHYLOGENETIC ANALYSES OF FAMILY GELIDIACEAE

A total of 59 *rbc*L sequences of family Gelidiaceae including 14 sequences of Malaysian specimens and three outgroups, *Gelidiella acerossa* (HM629846), *Gelidiella fanii* (HM026541) and *Pterocladia lucida* (LU01048) and 57 *cox*I sequences of Gelidiaceae including 14 sequences of Malaysian specimens and two outgroups, *Gelidiella acerosa* (HM102421) and *G. fanii* (HM026528) were used for phylogenetic analyses. The list of accession numbers of Gelidiaceae acquired from GenBank and obtained from Malaysian specimens has shown in Appendix 18. The length of *rbc*L and *cox*I gene obtained for the specimens of Family Gelidiaceae varied from 1375-1390bp (Table 4.1).

Twenty eight sequences of Malaysian species belong to Gelidiaceae including 14 sequences of *cox*I and 14 *rbc*L sequences were newly generated in this study. The phylogenetic matrix of *rbc*L data from 59 sequences consisting of 33 taxa of Gelidiaceae (including outgroups and 14 sequences of Malaysian specimens) included 1315 nucleotide of which 423 (32.1%) were parsimoniously informative, 824 (63%) constant and 68 (5%) parsimony-uninformative (Table 4.11).

In phylogenetic analyses of partial *cox*I gene 57 sequences including 14 newly obtained sequences from Malaysian specimens formed a matrix of 1200 nucleotide with 426 (35.5%) parsimoniously informative sites, 744 (68.3%) constant and 30 (2.5%) parsimony-uninformative (Table 4.11).

Table 4.11: Nucleotide composition and statistics for maximum parsimony and maximum likelihood analyses of the two sets of *rbc*L and *cox*I genes in phylogenetic analyses of Gelidiaceae.

	***rbc*L**	***cox*I**
Number of taxa	59	57
Nucleotides (base pair)	1315	1200
Base frequency (A/C/G)	0.319/0.148/0.203	0.341/0.105/0.121
Variable sites (%)	491 (32.1)	456 (38)
I (invariable site rate)	824(62.7)	744(62)
Informative sites (%)	355 (27)	426 (35.5)
Selected model	GTR + G +I	GTR + G +I
α (shape parameter)	0.2362	0.1091
Ln Likelihood	-91224.856	-9079.046

Sequences were straightforward necessitating neither introduction gaps nor ambiguous alignment possibilities. Separate phylogenetic analyses using ML, MP and BI methods gave similar phylogenetic trees but not congruent topologies; therefore only results from the ML analyses of each genetic marker were presented. Bootstrap values and percentage of posterior probabilities resulting from the three methods of analyses were appended on the branches of ML trees (Figs. 4.19 & 4.20).

The ML trees resulted from analyses of partial *rbc*L gene data of the 59 sequences comprised of 45 sequences of Gelidiaceae acquired from GenBank, and 14 sequences from Malaysia, and also 57 sequences of partial *cox*I gene including 14 sequences from Malaysian specimens did not support the monophyly of Gelidiaceae.

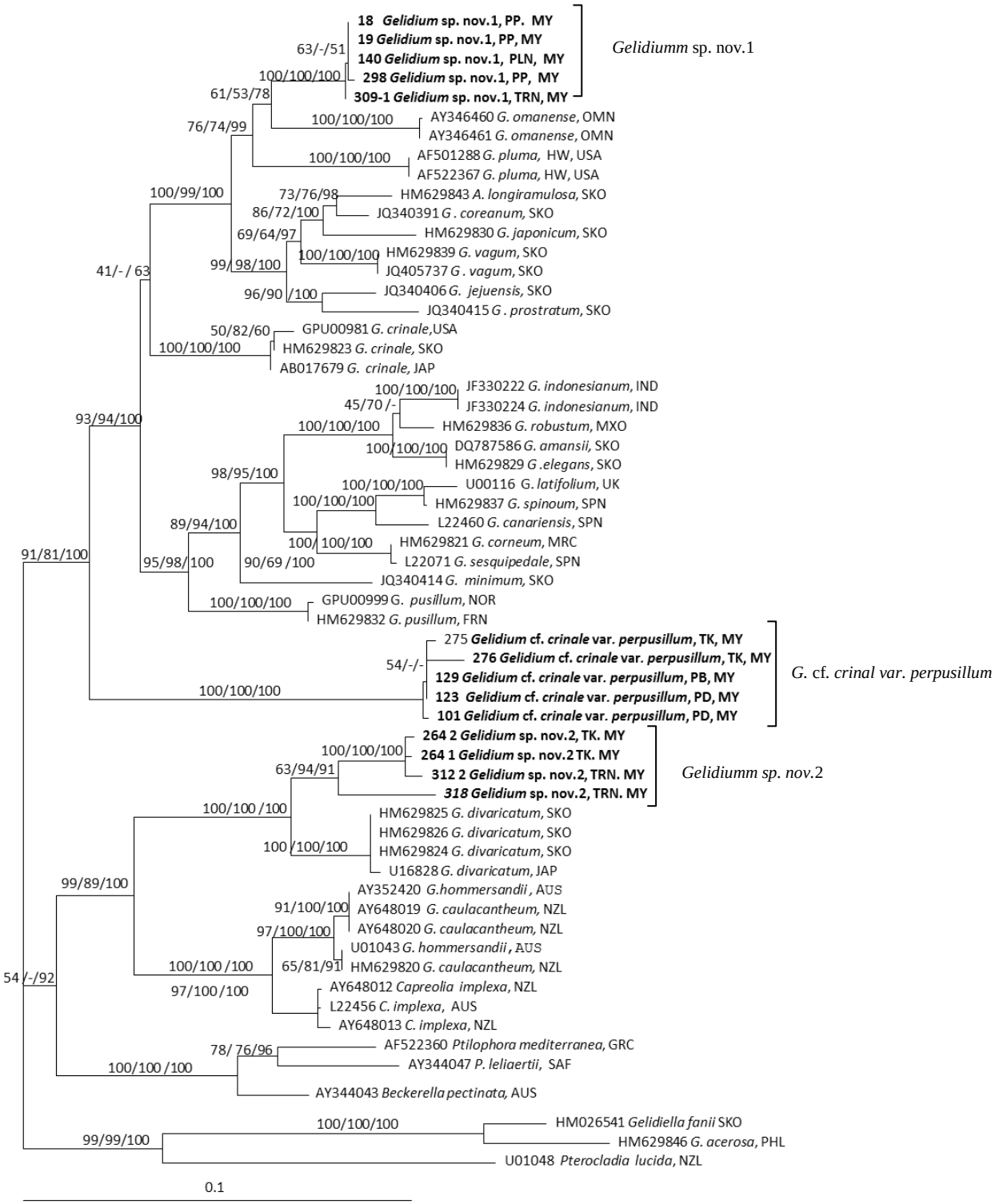

Figure 4.19: Maximum likelihood tree of 59 *rbc*L sequences of Gelidiaceae species including 14 sequences of Malaysian specimens. ML and MP bootstrap value and percentage of Bayesian inference probability appended over nodes. (AUS= Australia, FR= France, HW= Hawaii, IND=Indonesia, JAP= Japan, MRC=Morocco, MY= Malaysia, NCL= New Caledonia, NZL= New Zealand, OMN=Oman, PB=Pulau Besar, PD=Port Dickson, PP=Pulau Pinang PLN=Pulau Langkawi, PTR= Puerto Rico, SAF= South Africa, SKO= South Korea, SPN= Spain TRN=Terengganu, UK =United Kingdom, USA=United States of America).

Another clade Including *Capreolia implexa, G. divaricatum, G. caulacantheum* and *G. hommersandii* were separated from the main group of the *Gelidium* (*sensu lato*) species (Fig. 4.19), and in *cox*I analyses the clade comprised *G. divaricatum* and *G. caulacantheum,* was separated from the main group of *Gelidium* (Fig. 4.20).

All phylogenetic analyses using both genes, *rbc*L (Fig. 4.19) and *cox*I (Fig. 4.20), resolved the sequences of Malaysian specimens into three taxa which of them two taxa were not match with any known species of Gelidiaceae and proposed as two new species, *Gelidium* sp. nov. 1, and *Gelidium* sp. nov.2. The other one was proposed as new combination *Gelidium perpusillum* comb. nov. based on high morphological and ecological similarity with *G. crinale* var. *perpusillum* Piccone et Grunow.

In *rbc*L phylogenetic tree five specimens of *Gelidium* sp. nov.1, from Pulau Pinang, Pulau Langkawi, and Kuala Terengganu formed a monophyletic group with full support in all methods of analyses (Fig. 4.19). *Gelidium sp. nov.*1, showed sister relationship with *G. omanense* M. J .Wynne from Oman with weak to moderate support (Ml=61%, MP=53 & BI=78%) and grouped in a monophyletic clade with *G. pluma* Bornet ex N. H. Loomis from Hawaii, USA, with moderate to strong support in phylogenetic analyses (ML=76%, MP=74% & BI=99%). The *rbc*L sequence divergence among the sequences of *Gelidium sp. nov.*1 (0.0-0.1%) (Table 4.12) was within the range which is considered to be interspecific.. The divergence of the species from *G. omanense* (5.1-5.2%) and *G. pluma* (5.4-5.7%) (Table 4.12) distinguished the *Gelidium* sp. nov.1 from these species.

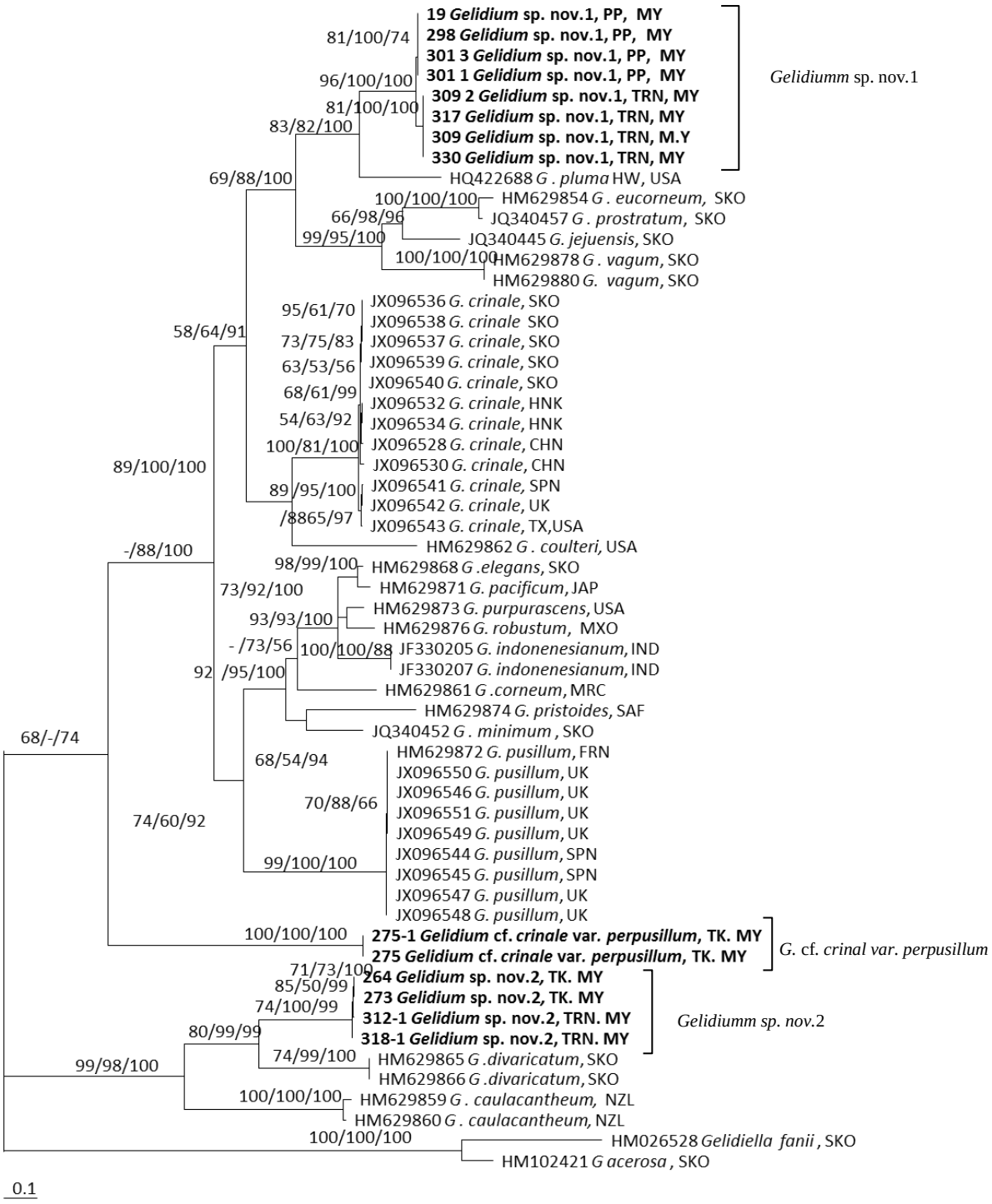

Figure 4.20: Maximum likelihood tree of 57 *coxI* sequences of Gelidiaceae species including 14 sequences of Malaysian specimens. ML and MP bootstrap value and percentage of Bayesian inference probability appended over nodes. (AUS= Australia, FRN= France, HNK=Hong Kong, HW= Hawaii, IND=Indonesia, JAP= Japan, MRC= Morocco, MXO= Mexico, MY= Malaysia, NZL= New Zealand, OMN=Oman, PP=Pulau Pinang, SAF= South Africa, SKO= South Korea, SPN= Spain, TK= Teluk Kemang, TRN=Terengganu, TX= Texas, UK = United Kingdom, USA=United State of America)

The *rbc*L sequence divergence of *Gelidium* sp. nov.1 (8.1-8.3%) from the specimens sequences of *Gelidium corneum* (Hudson) J. V. Lamouroux collected from type locality (Morraco) (Table 4.12) was in the interspecific divergence range of the genus *Gelidium.*

In the ML tree of *cox*I gene sequences, analyses of eight sequences of *Gelidium sp. nov.*1 collected from Pulau Pinang and Kuala Terengganu, formed a monophyletic group with strong support for ML (96%) and full support for MP and BI analyses methods (Fig. 4.20). In the *cox*I analyses, *Gelidium sp. nov.*1, showed sister relationship with *G. pluma* from Hawaii and formed monophyletic clade with a group comprising of *G. vagum, G. jejuensis, G. prostratum* from South Korea. The divergence of *cox*I gene sequences among the sequences of *Gelidium sp.nov*.1 (0.0-1.7%) was the level which represents same species. The sequence divergence of *cox*I gene of *Gelidium sp. nov.*1 from its sister species, *G. pluma* (9.3-9.7%) and from the species of sister clade (11.3-13.3%) showed the distinction of *Gelidium* sp. nov.1 from these species. *Cox*I sequences divergence of the species from *G. corneum* was 12.8-13.1%. (Table 4.13).

Five *rbc*L and two *cox*I sequences of the Malaysian specimens collected from roots and pneumatophores of mangrove grew near the rocky shores of Port Dickson, Pulau Besar and Teluk Kemang, which morphologically were similar to *G. crinale* (Turner) Gailon var. *perpusillum* Piccon & Grunow based on the illustrated picture in Dawson (1954). These sequences formed a distinct clade with full bootstrap values for all methods of analyses in both genes (Figs. 4.19 & 4.20) and did not show any close sister relationship with the sequences of *G. crinale* and other common *Gelidium* species (*sensue lato*) and also species of other genera, *Ptilophora, Capreolia,* in Gelidiaceae. These specimens did not match with other identified species based on molecular

analyses and proposed as new combination at species level in genus *Gelidium*. The sequences divergence of this Malaysian *Gelidium* (0.0-1.3% in *rbc*L and 0.0% in *cox*1) was in the range of the same species (Tables 4.12 & 4.13).

Divergence of the species from the type species of the family, *Gelidium corneum* (11.5-13.1% in *rbc*L and 14.7% in *cox*I) and from the species which were included in *Capreolia* clade (11.0-13.3% in *rbc*L and 14.2-16.2% *in cox*I) and *Ptilophora* clade (11.0-12.0 in *rbc*L) showed the species is a distinct species from all other species of the family, However this range of divergence represent the intergeneric divergence in the family, and in both *rbc*L and *cox*I genes phylogenetic trees sequences of this *Gelidium* grouped out of the main *Gelidium* clade and formed a new clade in the family Gelidiaceae (Figs.4.19 & 4.20).

Four sequences of *Gelidium* sp.nov.2 isolated from the populations of small seaweeds which grow as epizoic on the limpets attached to the rocks and artificial cement blocks in the intertidal zones of Teluk Kemang, and Kuala Terengganu, formed a distinct clade with weak to high support of *rbc*L analyses (ML=63%, MP=94% & BI=91%) and moderate to full support in *cox*I analyses (ML=74%, MP=100% & BI=99%) (Figs.4.19 & 4.20).

The species *Gelidium* sp. nov.2 showed sister relationship with *Gelidium divaricatum*, from Japan and South Korea with full support for all analyses using *rbc*L (Fig. 4.19), and moderate to full support (ML=80%, MP=99% & BI=99%) in *cox*I analyses (Fig. 4.20). These two species showed monophyly with a subclade comprising *G. caulacantheum* J. Agardh and *G. hommersandii* A. J. K. Millar & D. W. Freshwater reported from New Zealand and Australia and sequences of *Capreolia implexa* Guiry &

Womersley, which has been introduced as a biphasic genus in Gelidiaceae from New Zealand and Australia. The support value for this monophyletic clade was strong to full in *rbc*L analyses (ML=99%, MP=89% & BI=100%) (Fig. 4.19).

The pairwise divergence of the *Gelidium sp. nov.2* sequences (0.2-2.3% in *rbc*L) (Table 4.12) showed that the divergence level is a bit more than the intraspecific divergence, however the *cox*I divergence was low (0.2-0.7%) (Table 4.13), which represents the level of divergence for the same species. The sequences divergence of the species from its sister species, *G. divaricatum* (3.7-5.3% in *rbc*L & 12.2-12.6% in *cox*I) showed these two were distinct species. The sequences divergence of *Gelidium* sp.2 from the sequences of *G. caulacantheum* (8.1-9.4% in *rbc*L 14.3-14.4% in *cox*I) and *G. hommersandii* (8.1-9.1% in *rbc*L) (Tables 4.12 & 4.13) showed that all these species are within the range of the same genus, however all three *Gelidium* species in this monophyletic clade have triphasic life-cycle while life-cycle of *Capreolia implex w*as reported as biphasic (Guiry and Womersley, 1993).

Table 4.12: Pairwise distance between aligned sequences of partial *rbc*L region of Gelidiaceae species from Malaysia and species acquired from GeneBank, excluding gaps and ambiguities. Distance between taxon pairs calculated using uncorrected model. [Abbreviations for localities: AUS= Australia, HW=Hawaii, JAP=Japan MRC=Morocco, MY=Malaysia, NZL=New Zealand, OMN=Oman, SA= South Africa, SKO=South Korea].

		1	2	3	4	5	6	7	8	9	10	11
1	*Gelidium* sp.nov.1 (MY)	0.0-0.1										
2	*G. omanense* (OMN)	5.1-5.2	0.1									
3	*G. pluma* (HW)	5.4-5.7	6.6	0.0								
4	*G. perpusillum (MY)*	9.4-10-6	9.9-11.4	10.7-12.2	0.0-1.3							
5	*Gelidium* sp.nov.2 (MY)	10.9-11.7	11.7-12.5	12.4-13.4	11.1-11.9	0.0-2.3						
6	*G. divaricatum* (Jap & SKO)	10.9-11.6	11.4-12.2	11.8-12.6	11.4-12.5	3.4-4.5	0.0-0.3					
7	*G. caulacantheum* (NZL & AUS)	10.0-10.3	10.2-10.4	10.6-10.9	11.6-12.0	8.1-9.4	7.9-8.9	0.0-0.6				
8	*Capreolia. Imlexa*(AUS & NZL)	9.8-10.1	10.3-10.7	11.0-11.2	11.0-12.5	8.1-9.1	7.8-8.5	2.9-3.3	0.1-0.4			
9	*G. hommersandii* (AUS)	9.9-10.2	10.2-10.3	10.6	11.4-11.8	8.2-9.4	7.9-8.9	0.0-0.6	2.9-3.2	0.6		
10	*G. corneum* (MRC)	8.1-8.5	8.9-9.0	8.9	11.5-13.0	11.1-11.9	10.6-11.6	10.5-10.9	10.2-10.7	10-5-10.6	-	
11	*Ptilophora lelieart*i (SAF)	10.9-11.3	11.4	12.4	11.0-12.0	11.1-12.0	10.6-11.6	10.8-11.1	10.6-10.9	10.9-11.0	11.8	-

Table 4.13: Pairwise distance between aligned sequences of partial *cox*I region of Gelidiaceae species from Malaysia and species acquired from GeneBank, excluding gaps and ambiguities. Distance between taxon pairs calculated using uncorrected model [Abbreviations for localities: AUS= Australia, HW=Hawaii, JAP=Japan MRC=Morocco, MY=Malaysia, NZL=New Zealand, SA= South Africa, SKO=South Korea].

		1	2	3	4	5	6	7	8	
1	*Gelidium* sp.nov.1(MY)	0.0-1.7								
2	*G. pluma* (HW)	9.3-9.7	-							
3	*G. vagum* (SKO)	11.3-11.5	12.2	0.0						
4	*Gelidium perpusillum* (MY)	13.7-13.8	14.8	15.5	0.0					
5	*Gelidium* sp.nov.2(MY)	16.1-16.4	16.9-17.1	17.0-17.2	15.8-16.1	0.2-0.7				
6	*G. divaricatum* (Jap & SKO)	15.8-16.3	15.9-16.1	17.5-17.6	16.1-16.2	12.2-12.6	0.1			
7	*G. caulacantheum* (NZL)	15.7-15.8	15.3-15.7	15.5-16.3	14.2-14.6	14.3-14.4	14.1-14.4	2.4		
8	*G. corneum* (MRC)	12.8-13.1	12.7	13.8	14.7	16.3-16.5	16.0-164	16.2-16.3	-	
9	*G. crinale* (SKO, UK, US, CHN, HNK)	12.6-14.0	15.1-15.6	12.1-13.3	15.1-15.4	18.0-19.2	17.8-194	16.2-17.2	14.1-14.4	0.1-2.3

4.2.4 PHYLOGENETIC ANALYSES OF FAMILY GELIDIELACEAE

A total of 29 *rbc*L sequences of family Gelidiellaceae including nine sequences of Malaysian specimens and four outgroups, *Pterocladiella capillacea* (AB023850), *Pterocladia lucida* (AY648025) and *Ptilophora leliaertii* (AY344047) and *Gelidium corneum* (HM629821), and 16 *cox*I sequences of Gelidiellaceae including seven sequences of Malaysisn specimens and two outgroups, *Gelidium caulacantheum* (HM629860) and *G. corneum* (HM629861) were used for phylogenetic analyses. The list of sequences of Gelidiellaceae extracted from GenBank and resulted from Malaysian specimens is shown in Appendix 19.

15 sequences of Malaysian species belong to Gelidiaceae including nine *rbc*L and six *cox*I sequences were generated in this study. The phylogenetic matrix of *rbc*L data from 29 sequences consisting of 10 taxa of Gelidiales (including nine sequences from Malaysian specimens) included 1162 nucleotides with 280 (24.1%) parsimoniously informative site, 790 (68%) constant and 92 (7.9%) parsimony-uninformative (Table 4.14). Sequences were straightforward necessitating neither introduction gaps nor ambiguous alignment possibilities. Separate phylogenetic analyses using ML, MP and BI methods gave constructed similar phylogenetic tree but not congruent topologies. For summary only results from the ML analyses of each genetic marker were presented. Bootstrap values and percentage of posterior probabilities resulting from the three methods of analyses were appended on the branches of ML phylogeny (Figs. 4.21 & 4.22).

In phylogenetic analyses of partial *cox*I gene for six newly obtained sequences from Malaysian specimens of Gelidiellaceae along with 9 sequences acquired from Genebank,

formed a matrix of 600 nucleotides with 142 (35.5%) parsimoniously informative sites, 410 (68.3%) constant and 48 (8%) parsimony-uninformative (Table 4.14).

Table 4.14: Nucleotide composition and statistics for maximum parsimony and maximum likelihood analyses of the two sets of *rbc*L and *cox*I genes in phylogenetic analyses of Gelidiellaceae.

	***rbc*L**	***cox*I**
Number of taxa	32	15
Nucleotides (base pair)	1162	600
Base frequency (A/C/G)	0.311/0.143/0.210	0.285/0.149 /0.169
Variable sites (%)	372 (32.0)	190 (31.7)
I (invariable site rate)	790 (68)	410 (68.3)
Informative sites (%)	280 (24.1)	142 (8)
Selected model	GTR + G +I	J2+G+I
α (shape parameter)	0.2265	0.1679
Ln Likelihood	-5615.418	-2019.27

The ML trees from phylogenetic analyses of partial *rbc*L gene data of 32 sequences of Gelidiellaceae, comprised of 23 sequences acquired from GenBank, and nine sequences obtained from Malaysia specimens, and also 15 sequences of partial *cox*I gene including six sequences from Malaysian specimens, grouped the sequences of the family in two main clades comprising *Gelidiella* and *Parviphycus* clades (Figs. 4.21 & 4.22).

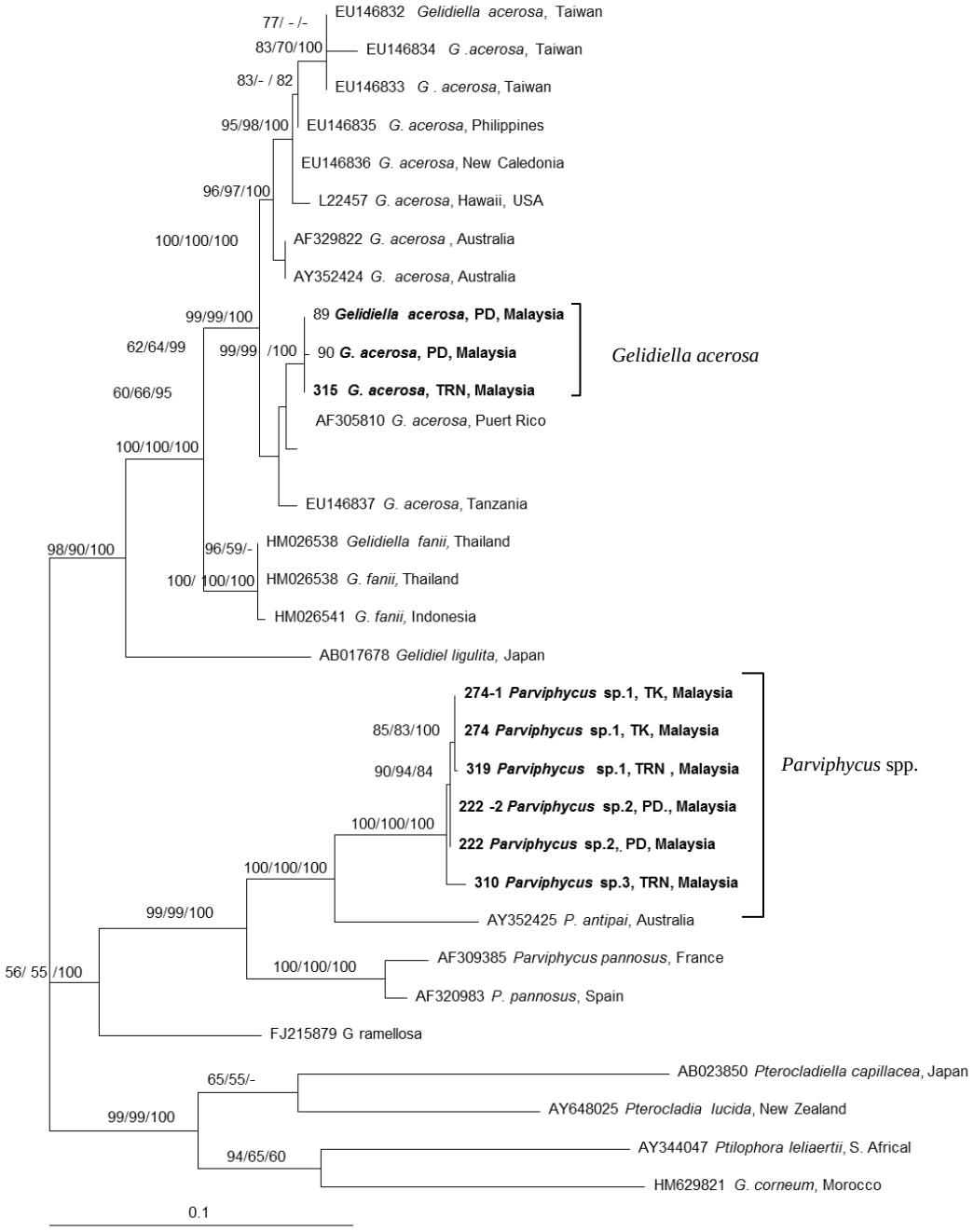

Figure 4.21: Maximum likelihood tree of 32 *rbc*L sequences of Gelidiellaceae including nine sequences of Malaysian specimen. ML and MP bootstrap value and percentage of Bayesian probability are shown for each clade over the clade node. (PD= Port Dickson, TRN=Terengganu, TK= Teluk Kemang).

Three Malaysian sequences of *Gelidiella acerosa* collected from Port Dickson and Kuala Terengganu in *rbc*L gene data analyses formed a distinct group with strong to full support (ML=99%, MP=99% & BI=100%) (Fig.4.21). The group of Malaysian sequences of *Gelidiella acerosa*, with full support for all analyses resolved within the sequences of *Gelidiella acerosa* which were reported from America, Africa, Australia, Hawaii Taiwan, Philippines and New Caledonia. Malaysian specimens of *G. acerosa* showed sister relationship with the sequences of the species from Puerto Rico and Costa Rica, South America and grouped with one sequence of species from Tanzania, eastern Africa, with weak to high support (ML=62%, MP=64% & BI=99%) and sequences of *G. acerosa* from Taiwan and Philippines were grouped with the sequences of the species from Australia, New Caledonia and Hawaii with strong to full support (ML=96%, MP=97% & Bi=100%) (Fig. 4.21).

Intraspecific *rbc*L sequences divergence of Malaysian *G. acerosa* (0.0-0.1%) was the level represent same species. Divergence of Malaysian sequences from sequences of species from South America and East Africa (1.1-1.5%), from the species of Australia, Hawaii and New Caledonia (2.3-3.3%) and from species of Philippines and Taiwan (2.8-4.6%) (Table 4.15), showed Malaysian species of *G. acerosa* genetically is closer to the South American and eastern African species than to the species from Australia, New Caledonia, Hawaii, Taiwan and Philippines (Fig. 4.21).

In analyses of *cox*I gene sequences the sequences of Malysian *G. acerosa* in the absence of South American, eastern African and Australian species, grouped within the

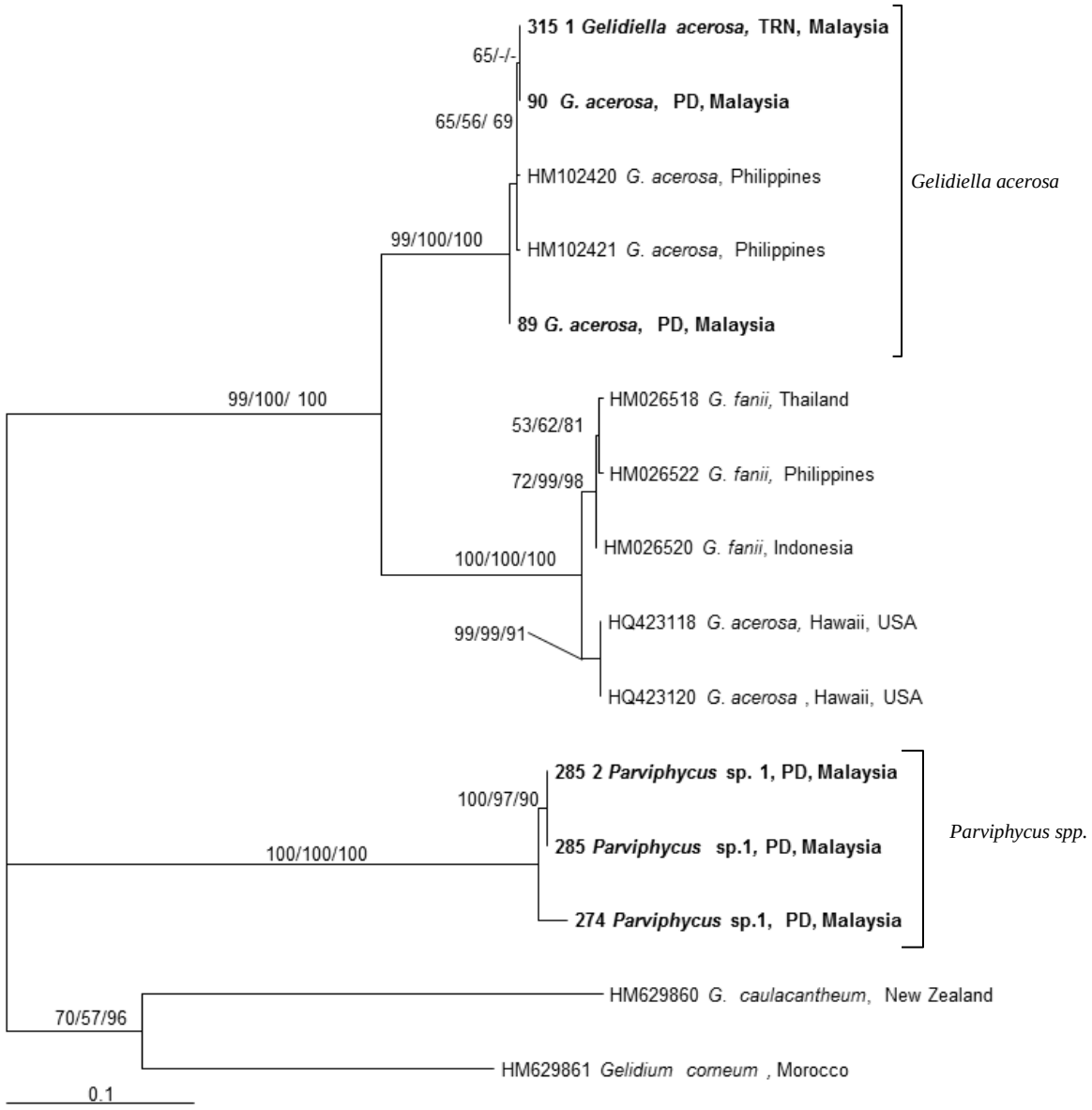

Figure 4.22: Maximum likelihood tree of 15 *cox*I sequences of Gelidiellaceae including six sequences of Malaysian specimen. ML and MP bootstrap value and percentage of Bayesian probability were appended over the clade node. (PD= Port Dickson, TRN=Terengganu, TK= Teluk Kemang).

sequences of species from Philippines with strong to full support (ML=99%, MP=100% & BI=100%) (Fig. 4.22). In *cox*I gene analyses, the sequences of Hawaiian *G. acerosa* reported by Sherwood *et al.* (2010) were grouped with *Gelidiella fanii* from Thailand and Indonesia. Pairwise divergence of *cox*I gene sequences among Malaysian specimens of *G. acerosa* (0.0-0.5%) and their divergence from the sequences of *G. acerosa* in Philippines (0.2-0.5%) were in the range represent same species (Table 4.16). Pairwise sequence divergence between Malaysian sequences of *G. acerosa* and Hawaiian sequences (11.9-12.5%) was similar to the divergence of the Philippines species of *G. acerosa* from the Hawaiian species (12.6%) which were similar to the divergence of *G. fanii* from *G. acerosa* (11.1-12.3%) showing that *G. acerosa* reported from Hawaii (Sherwood *et al.*, 2010) should belong to *G. fanii*.

The other groups of Malaysian Gelidiellaceae included the specimens of genus *Parviphycus*, which grouped with strong to full support for both *rbc*L and *cox*I genes analyses in the clade contain *Parviphycus* species (Figs. 4.21 & 4.22).

In phylogenetic analyses of partial *rbc*L gene sequences, six sequences of Malaysian specimens formed a monophyletic subclade with full support for all methods of analyses (Fig. 4.21). The group showed a sister relationship with *Parviphycus antipai* reported from Australia with full support in all analyses. *P. pannosus* from Spain and France reported by Rico *et al.* (2002) showed monophyly with strong to full support (ML=99%, MP= 99% & BI=100%) with the group containing Malaysian *Parviphycus* and *P. antipai* from Australia. One sequence *Gelidiella ramellosa* from Australia also grouped in the *Parviphycus* clade (Fig. 4.21), however in analyses of LSU and *rbc*L sequences by Huisman *et al*. (2009) the

phylogenetic position of *G. ramellosa* was reported to be equivocal between *Gelidiella* and *Parviphycus.*

Pairwise divergence of six *rbc*L sequences from Malaysian *Parviphycus* (0.0-1.0%) showed these specimens were in the intraspecific level. Pairwise divergence of Malaysian *Parviphycus* sequences from their sister species, *P. antipai* (7.2-7.5%) and from *P. pannosus* (9.1-10.7%) and also from *Gelidiella ramellosa* (10-6-12.0%) (Table 4.22) showed the Malaysian specimens of *Parviphycus* are distinct from all these three species (Fig. 4.21). In spite of phylogenetical similarity among the Malaysian species of *Parviphycus*, these specimens showed different morphological features which can places them in three groups. In the phylogenetic tree of *rbc*L the three species of *Parviphycus* from Malysia were shown to be different but not highly supported (Fig. 4.21)

Three sequences of the *Parviphycus* Sp.1 specimens collected from Teluk Kemang, in west coast and Pantai Bukit Kelung, Kuala Terengganu in east coast which morphologically were very similar were grouped together (Fig.4.21). The two sequences of *Parviphycus* sp.2 collected from Port Dickson are from same population, while *Parviphycus* sp.3 with very different morphology was collected from Pantai Chendering, Kuala Terengganu. These morphological variations despite genetic similarity showed the need more study before any decision is made about species status.

The pairwise divergence among *rbc*L sequences of the genera *Parviphycus* and genus *Gelidiella* (10-15.3%) (Table 4.15) was more than the intergeneric divergence of family Gelidiaceae containing heterogenetic genera which was maximum up to 11.5 -13% (Table. 4.12).

In *cox*I gene analyses three sequences of *Paviphycus* sp.1 formed a new clade in the *cox*I gene phylogenetic tree of Gelidiellaceae with full support for all methods of analyses (Fig. 4.22). These three specimens also were collected from Port Dickson and were morphologically very similar. The pairwise sequences divergence in the *cox*I gene (0.0-1.7) was the the level of same species (Table 4.16).

The pairwise divergence of *cox*I sequences of *Parviphycus* sp.1 from the sequences of *Gelidiella* species acquired from GenBank including, *G. acerosa* from Hawaii (20.3-20.8%), *G. acerosa* from Philippines (18.3-18.7%), *G. acerosa* from Malaysia (18.2-19.4%) and *G. fanii* from Philippines (20.4-20.9) were very high (Table 4.16) and raised the question about the erection of a new family to accommodate of these distant species. The need for a new family may be more necessary when the level of divergence is compared with the divergence of *Gelidium corneum*, type species of family Gelidiaceae, from the *Gelidiella acerosa*, type species of family Gelidiellaceae, which was 19.6-20.1% that is lower than the divergence of Malaysian specimen *Parviphycus* from *Gelidiella acerosa* from Hawaii (20.3-20.8%).

These analyses also showed the divergence between *Gelidium caulacantheum* and *G. corneum* (18.7%) (Table 4.16) which taxonomically are in same family, is very high and there is a need for more study to clarify the position of different genera in Gelidiaceae as well as Gelidiellaceae and Pterocladiaceae.

Table 4.15: Pairwise distance between sequences of partial *rbc*L region of the species of Gelidiellaceae from Malaysia and the sequences acquired from GenBank acquired, excluding gaps and ambiguities. Distance between taxon pairs calculated using uncorrected model. [Abbreviations for localities: AUS=Australia, CAR=Caribbean, EAF=Eastern Africa, JAP=Japan, EU=Europe, HW=Hawaii, MY=Malaysia, NC=New Caledonia,PHL=Philippines]

		1	2	3	4	5	6	7	8	9	10	11	12
1	*G. acerosa* (CAR, EAF)	0.3-1.4											
2	*G. acerosa* (HW, AUS & NC)	2.2-3.3	0.0-1.7										
3	*G. acerosa* (PHL & TAI)	2.4-4.4	0.2-3.1	0.0-1.9									
4	*G. fanii* (TH & PHL)	4.4-4.8	4.6- 6.3	0.2-3.1	0.0-0.3								
5	*G. acerosa* (MY)	1.1-1.5	2.3-3.3	2.8-4.6	4.0-4.4	0.0-0.1							
6	*G. .ligulata* (JAP)	8.3-8.6	8.9-9.6	8.1-9.6	8.2-8.4	8.5-8.6	-						
7	*G. ramellosa* (AUS)	11.1-11.2	10.8-11.6	12.0-13.5	10.0-10.1	11.1-11.2	10.5	-					
8	*Parviphycus pannosus* (EU)	12.3-12.8	12.4-13.2	12.0-14.3	11.2-11.4	12.5-13.0	12.7	10.6-11.0	2.0				
9	*P.antipai* (AUS)	12.9-13.0	12.9-13.1	12.9-15.3	12.1-12.2	13.4-13.5	14.1	11.8	9.9-10.7	-			
10	*Parviphycus* sp.1 (MY)	12.6-13.7	13.0-13.3	13.2-15.3	12.0-12.2	12.7-13.0	13.2	11.9-12.0	9.2-9.8	7.3	0.0-0.1		
11	*Parviphycus* sp.2 (MY)	12.4-13.4	12.8-13.0	13.3-14.9	12.0-12.1	12.6-12.7	13.0	11.8	9.1-9.6	7.2	0.1-0.2	0.0	
12	*Parviphycus* sp.3 (MY)	13.0-14.0	13.4-13.6	13.5-15.1	12.4-12.5	13.2-13.3	12.5	11.8-12.0	9.5	7.5	0.9-1.0	0.7	-

Table 4.16: Pairwise distance between different aligned sequences of partial *cox*I region of the species of Gelidiellaceae from Malaysia and the sequences acquired from GeneBank, excluding gaps and ambiguities. Distance between taxon pairs calculated using uncorrected model. [Abbreviations for localities: HW=Hawaii, MY= Malaysia, NZL=New Zealand, MRC=Morocco, PHL=Philippines].

		1	2	3	4	5	6	7
1	*Gelidiella acerosa* (HW)	0.0						
2	*Gelidiella acerosa* (PHL)	12.6	0.3					
3	*G. fanii* (TH)	1.7-2.1	11.1-12.3	0.3				
4	*G. acerosa* (MY)	11.9-12.5	0.2-0.5	11.2-12.1	0.0-0.5			
5	*Parviphycus sp*. (MY)	20.3-20.8	18.3-18.7	20.5-21.5	18.2-19.4	0.0-1.7		
6	*Gelidium caulacantheum* (NZL)	12.6	19.7-21.2	20.4-20.9	19.9-20.0	20.7-21.2	-	
7	*G. corneum* (MRC)	20.0	19.6-20.1	19.6-20.0	19.9-20.3	19.9-20.9	18.7	-

4.2.5 INTERFAMILIAL RELATIONSHIPS OF ORDER GELIDIELES

For evaluation of the taxonomic position of Malaysian Gelidiales and finding an overall phylogeny in this order, sequences of all Malaysian species for three markers *rbc*L, *cox*I and LSU, together with most sequences of members of this order available in the GenBank were used for comprehensive analyses in all three genetic markers.

151 sequences of partial *rbc*L gene (including 44 new sequences from Malaysia specimens), 89 sequences of *cox*I gene (including 44 sequences of newly obtained from Malaysian species) and 56 sequences of LSU (with 9 new sequences of Malaysia specimen) were used for overall analyses of order Gelidieles. Nucleotide composition and statistics for maximum parsimony and maximum likelihood analyses of the three sets of *rbc*L, *cox*I and LSU genes in phylogenetic analyses of Gelidiales are shown in Table 4.17

Table 4.17: Nucleotide composition and statistics for maximum parsimony and maximum likelihood analyses of the three sets of Gelidiales sequences.

	***rbc*L**	***cox*I**	**LSU**
Number of taxa	151	89	56
Nucleotides (base pair)	1145	546	1129
Base frequency (A/C/G)	0.346/0.142/0.142	0.348/0.108/0.095	0.251/0.206/0.292
Variable sites (%)	243 (21.2)	242(44.3)	224(19.8)
I (invariable site rate)	902(78.7)	304(55.7)	905(80.1)
Informative sites (%)	180 (15.7)	223 (40.8)	71(6.3)
Selected model	J3 + G +I	GTR+G+I	TVM+G
α (shape parameter)	0.2177	0.1045	0.1163
Ln Likelihood	-13786.11	-6703.564	-3926.95

For these analyses three methods, maximum likelihood, maximum parsimony and Bayesian analyses were applied for all three set of sequences. Sequences of Gigartinales and Rhodymeniales were used as outgroups. The resultant trees were presented in Figures 4.23, 4.24 and 4.25 and their mean pairwise divergence has shown in Tables 4.18 to 4.20.

In these analyses, species of Gelidieaceae with the highest number of sequences in GenBank formed distinct groups in all three phylogenetic trees (Figs. 4.23- 4.25). Malaysian species of Gelidiaceae were grouped in two clades of this family. *Gelidium* sp.nov.1 and *Gelidium* cf. *crinale* var. *perpusillum* were grouped in common clade of *Gelidium* and *Gelidium* sp. nov.2 was classified in clade of *Capreolia*. Results of these analyses indicated two groups *Capreolia* and *Ptilophora* did not show monophyly with the large clade of *Gelidium* based on two gene *rbc*L (Fig. 4.23) and LSU (Fig 4.25). In *cox*I analyses of order Gelidiales there were no sequences of *Ptilophora* and *Capreolia* in GeneBank, but sequences of *G. caulacantheum*, *G. divaricatum* and *Gelidium* sp.nov.2 from Malaysia, formed a distinct subclade in the *Gelidium* clade, and all together showed sister relationship with *Gelidium* cf. *crinale* var. *perpusillum* from Malaysia (Fig. 4.24).

The clade with mainly species of *Gelidiella* formed the basal group supported with high to full value (ML=94%, MP=96% & BI=100%). The other members of Gelidiellaceae, *Parviphycus*, were shown to have sister relationship with Pterocladiaceae with full support for all analyses (Fig. 4.24).

22 species of Pterocladiaceae collected from Malaysia all grouped in the clade Pterocladiaceae and formed two new subclades in the clade *Pterocladiella* which were

proposed as two new species *Pterocladiella* sp.nov.1 and *Pterocladiella* sp.nov.2. Two groups of collected specimens from Malaysia belong to the genus *Pterocladiella* were two new records. One group was solved in *P. bartlettii* and the other group of sequences resolved in the clade composed of *P.beachiae*, which showed sister relationship with *P. australafricanensis.*

Another group of *Pterocladiella* was resolved in subclade *P. caerulescens* which was already reported from Malaysia (Fig. 4.23). Two sequences of Malaysian specimens (whose attachment system showed similarity with *Aphanta pachyrrhiza* from South Africa) were grouped with sequences of *Aphanta pachyrrhiza* but due to high divergence (10.0 % in *rbc*L, 2.3% in LSU) and some morphological difference showed they are two distinct species.

The position of the genus *Aphanta* in Gelidiales was ambiguous when the genus was introduced based on molecular analyses of *rbc*L and LSU genes (Tronchin & Freshwater (2007). The general analyses based on three genes *rbc*L, *cox*I and LSU in our study showed the sequences of African and Malaysian species of *Aphanta* formed distinct individual subclades (Figs. 4.23, 4.24 & 4.25).

Analyses of *cox*I gene sequences obtained from Malaysian specimens of *Aphanta* showed this group is distant from Pterocladiaceae (Fig. 4.24). Mean pairwise divergence of South African and Malaysian sequences *Aphanta* from members of Pterocladiaceae (12.2% in *rbc*L, 15.9% in *cox*I, and 3.7% in LSU), *Pterocladia lucida* (13.5% in *rbc*L, and 4.0% in LSU), *Gelidium* (13.1% in *rbc*L, 16.6% in *cox*I and 4.0 % in LSU), *Gelidiella* (14.9% in *rbc*L, 16.9% in *cox*I, and 4.9 % in LSU) and *Parviphycus* (13.4%

in *rbc*L, 17.7% in *cox*I, and 3.8 % in LSU) (Tables 4.18 - 4.20, these levels of divergence are similar to the divergence between two families Pterocladiaceae and Gelidiaceae, which means two clades *Aphanta* and *Pterocladia* have the potential to be elevated to family level, however there is a need for more morphological studies and molecular analyses.

Family Gelidiellaceae with two main clades *Gelidiella* and *Parviphycus* also showed monophyly in *rbc*L and LSU analyses but were paraphyletic in *cox*I gene analyses. Sequences of *cox*I gene obtained from Malaysian *Parviphycus* showed monophyly with the family Pterocladiceae with weakly to strong support for Ml (58%), BI (94%) and no support in MP analysis. Most of the previously reported sequences of *Gelidiella* were grouped in a same clade except *Gelidiella ramellosa* which in two analyses based on *rbc*L and LSU genes showed monophyly with genus *Parviphycu*s (Figs. 4.23 & 4.25).

The morphological characters of *G. ramellosa* explained by Huisman *et al.* (2009) are more similar to genus *Parviphycus* than *Gelidiella.* Sequences of c*ox*I gene for genus *Parviphycus* were produced for the first time in this study and there was no *cox*I gene sequence of the genus in GenBank for comperative purpose. Pairwise divergences of this clade from other groups of Gelidiales were in the level that shows the need for more study to be erected to family level (Table 4.23 and 4.24) however more study is needed to clarify the phylogenetic position of this genus.

The interfamilial relationship among the Pterocladiaceae , Gelidiaceae and Gelidiellaceae is explained based on the *rbc*L phylogentic tree (Fig. 4.23). Pterocladiaceae is not a monophyletic family and *Pterocladia lucida* as type species

of the family shows paraphyletic relationship with all the species of the family. The *Aphanta* sp. from Malaysia and *A. pachyrrhiza* from South Africa also showed paraphyletic relationship with other species of Pterocladiaceae. In this analyses Pterocladiaceae showed closer relationship with Gelidiellaceae than Gelidiaceae. *Gelidium, Ptilophora* and *Capreolia* (including *Capreolia implex, G. divaricatum, G. hommersandii, G. caulacantheum and Gelidium* sp.2 from Malaysia) formed three distinct clades in this family but all three clades, *Gelidium, Capreolia and Ptilophora* originated from the same ancestors.

In cox1 analyses, the genus *Aphanta* showed paraphyletic relationship with other species of Pterocladiaceae and formed a new clade between *Gelidiellaceae* and *Gelidiaceae* (Fig 4.24). *Parviphycus* sp. from Malaysia also showed paraphyletic relationship with *Gelidiella* and formed a new clade between *Gelidium* and *Pterocladia*. In this analysis *Gelidiella* from the family Gelidiellaceae formed the basal clade of the phylogenetic tree and *Gelidium* showed more evolutionary changes than Gelidiellaceae and Pterocladiaceae. In the LSU phylogenetic tree two clades *Ptilophora* and *Gelidium* did not originate from the same ancestor. The *Capreolia* clade showed weak monophyly with two other families Pterocladiaceae and Gelidiellaceae.

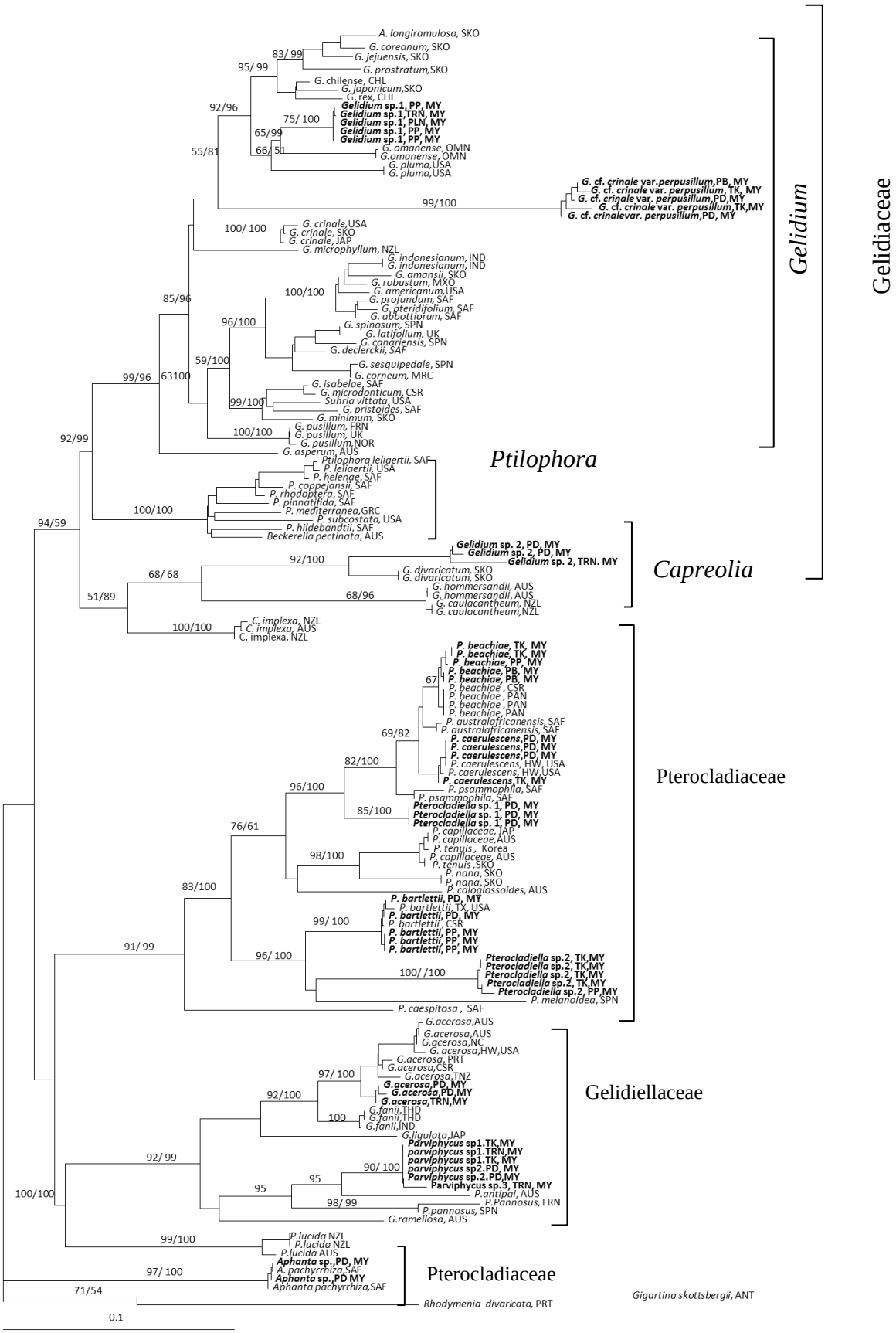

Figure 4.23: Maximum likelihood tree of 151 *rbc*L sequences of Gelidiales species including 44 sequences of Malaysian specimens. ML and MP bootstrap value are respectively shown for each clade over the clade node. (ANT=Anatctica, AUS= Australia, CHL=Chile, CSR= Costa Rica, FRN= France, HW= Hawaii, IND=Indonesia, JAP= Japan, MRC= Morocco, MY= Malaysia, NC= New Caledonia, NZL= New Zealand, NRW= Norway, OMN=Oman, ITL=Italy, PD=Port Dickson, PP=Pulau Pinang, PLN=Pulau Langkawi, PRT= Puerto Rico, GRC=Greec, SAF=South Africa TAN=Tanzania, THD=Thailand, TK=Teluk Kemang, TRN= Terengganu, TX=Texas, VNZ=Venezuela).

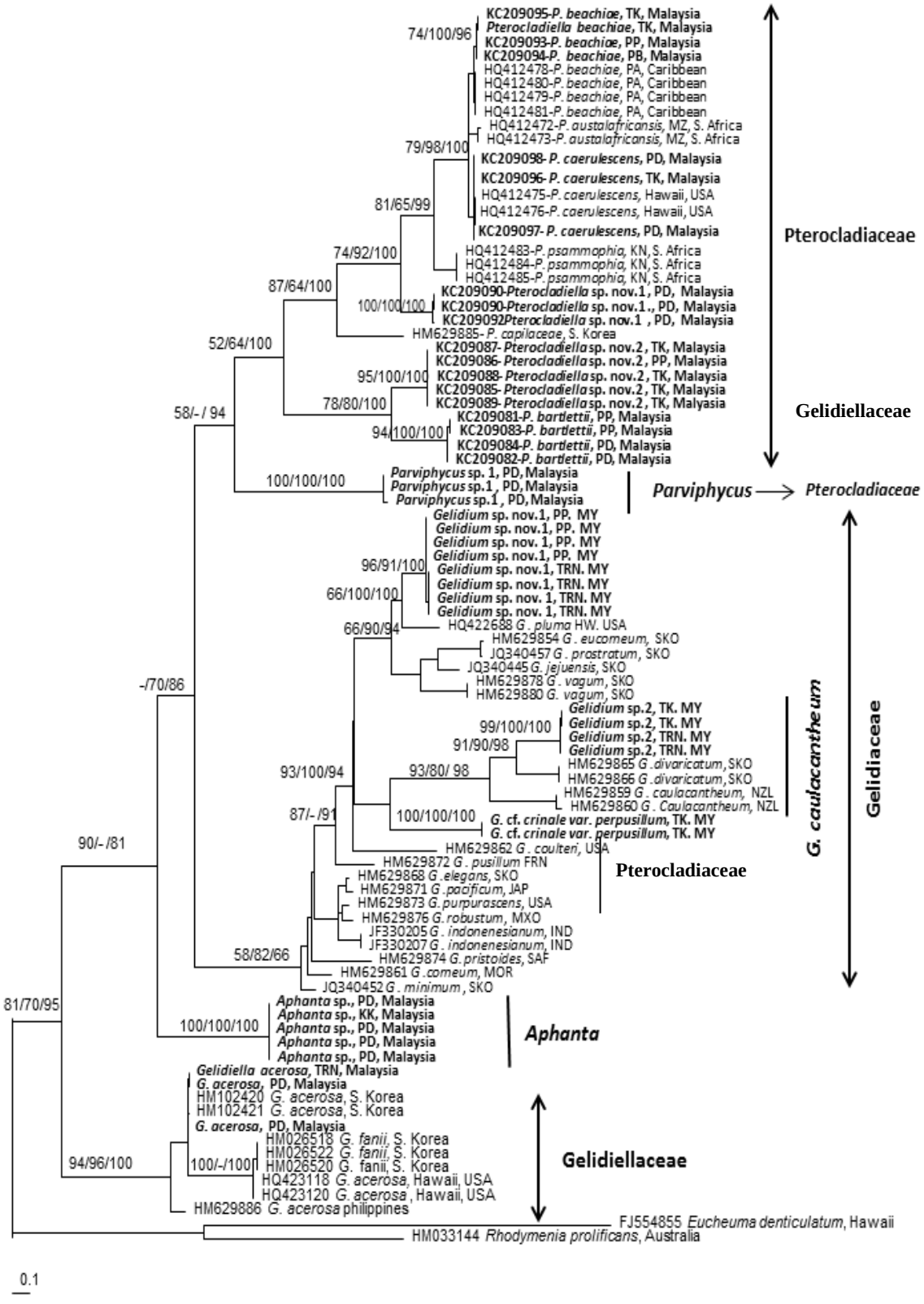

Figure 4.24: Maximum likelihood tree of 89 *coxI* sequences of Gelidiales species including 44 sequences of Malaysian specimens. ML and MP bootstrap value and percentage of posterior of BI are shown for each clade over the clade node. (AUS= Australia, CSR= Costa Rica, FRN= France, HW= Hawaii, IND=Indonesia, JAP= Japan, KK=Kota Kinabalu, KN=Kwazulu-Natal, MOR= Morocco, MY= Malaysia, MXO= Mexico, MZ=Mozambiqu, NC= New Caledonia, NZL= New Zealand, NRW= Norway, PA=Panama, PB=Pulau Besar, PD= Port Dickson, PP=Pulau Pinang, PTR=Puerto Rico, TAN=Tanzania, TK=Teluk Kemang, TRN=Terengganu, SAF=South Africa, SKO=South Korea, USA= United States of America).

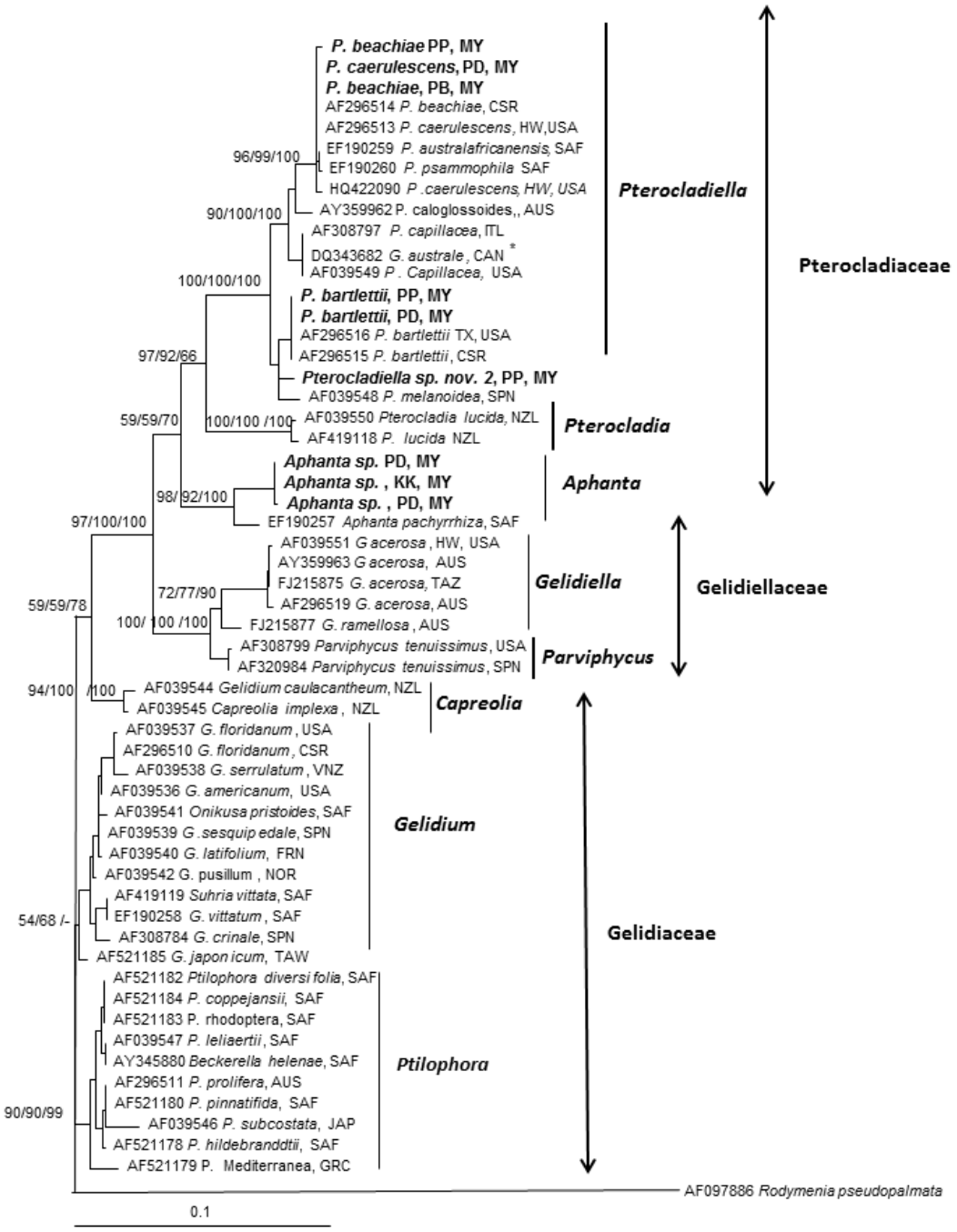

Figure 4.25: Maximum likelihood tree of 56 LSU sequences of Gelidiales species including 9 sequences of Malaysian specimens. ML and MP bootstrap value and percentage of posterior support of BI are shown for each clade over the clade node. [AUS= Australia, CAN=Canada, CSR= Costa Rica,FRN= France, GRS=Greec, HW= Hawaii, IND=Indonesia, ITL=Italy, JAP= Japan, KK=Kota Kinabalu, MRC= Morocco, MY= Malaysia, NC= New Caledonia, NZL= New Zealand, NOR= Norway, PD= Port Dickson, PP=Pulau Pinang, PTR= Puerto Rico, SAF=South Africa, SPN=Spain, TAN=Tanzania, , USA= United States of America, VNZ=Venezuela, * sign shows the *G. austral* (DQ343682) is grouped in sequences of *P. capillacea*].

Table 4.18: Mean pairwise divergences for *rbc*L sequences order Gelidiales.

		1	2	3	4	5	6	7	8	9
1	Pterocladiaceae	7.7								
2	*Pterocladia lucida*	12.4	1.1							
3	*Aphanta*	12.2	13.5	6.1						
4	*Gelidium*	10.9	13.4	13.1	8.5					
5	*Capreolia*	9.8	12.7	11.6	10.1	4.1				
6	*Ptilophora*	12.4	14.2	13.5	13.9	10.0	3.7			
7	*Gelidiella*	11.4	14.9	14.6	13.2	13.9	13.5	4.0		
8	*Parviphycus*	11.8	15.0	13.4	14.1	13.6	14.9	11.7	5.9	
9	Outgroup	14.8	15.1	16.5	15.6	13.8	16.9	17.3	17.0	16.6

Table 4.19: Mean pairwise divergences for *cox*I sequences order Gelidiales.

		1	2	3	4	5	6	7
1	*Pterocladia*	10.6						
2	*Aphanta* sp. Malaysia	15.9	0.2					
3	*Gelidium*	17.2	16.6	11.9				
4	*G.caulacantum*	18.5	17.5	16.8	10.4			
5	*Gelidiella*	18.5	16.9	17.6	18.3	7.2		
6	*Parviphycus*	16.9	17.7	17.1	19.3	17.7	1.3	
7	Outgroup	18.4	20.2	19.8	20.8	19.2	18.7	20.2

Table 4.20: Mean pairwise divergences for LSU sequences order Gelidiales.

		1	2	3	4	5	6	7		
1	*Pterocladia*	0.9								
2	*Pterocladia lucida*	4.0	0.5							
3	*Aphanta*	3.8	4.0	0.7						
4	*Gelidium*	4.8	3.5	4.0	0.1					
5	*Capreolia*	5.3	3.0	4.4	1.3	0.3				
6	*Ptilophora*	5.2	3.4	4.5	1.4	1.3	0.5			
7	*Gelidiella*	6.4	5.8	4.9	4.8	4.9	5.5	0.7		
8	*Parviphycus*	4.8	4.2	4.0	3.8	4.1	4.5	1.7	0.0	
9	Outgroup	11.3	10.2	10.4	8.6	8.5	8.9	10.6	10.1	-

5.0 DISCUSSION

5.1 Gelidiales from Malaysia

One hundred and twenty four specimens were collected from ten localities in Malaysia. They were identified as 13 morphospecies belonging to three families and five genera. Molecular analyses separated the collection into 11 species in the three families and five genera. Of these four are proposed as new species, one as new combination and two are new records for Malaysia.

5.2 FAMILY PTEROCLADIACEAE

5.2.1 *Pterocladiella bartlettii* (Taylor) Santelices

Branching pattern, form of erect branches, entangled and bilateral long divaricate ascending branches, blackish colour and habit of the collected specimens of *P. bartlettii* from Port Dickson, Malaysia, agreed well with the original type description of *P. bartlettii* (Taylor, 1943). However, the specimens from Pulau Pinang showed some differences in habit and colour and their size was smaller (Figs 4.3B & 4.3D) and also some features of Malaysian specimens, such as axially expanded tetrasporangial sori and low number of rhizines were more similar to *P. melanoidea* (Bárbara and Tapia, 2012, Dixon and Valéra, 1961). Thomas and Freshwater (2001) mentioned that their samples collected from Cahuita, Casta Rica (AF308506) were similar to *P. melanoidea var. gracilis* (Feldman & Hamel) Wynne, but their anatomical features revealed more similarity to *P. bartlettii* (Thomas and Freshwater, 2001).

Anatomical studies of the Malaysian specimens of *P. bartlettii* provided detailed unique data from vegetative and tetrasporangial sori structure. Analyses of new molecular data of *rbc*L and *cox*I genes of the species achieved in this study, combined

with the detailed morphological data revealed that detailed morphological study can reflect the phylogenetic evolution. *P. bartlettii* and *P. melanoidea* show some synapomorphy in branching pattern, intercalary tetrasporangial sori, and low number of rhizines and periclinal arrangement of cortical cells in transverse section of the stolon (Bárbara and Tapia, 2012).

Molecular analysis of *rbc*L and LSU genes resolved all specimens with strong to full support in *P. bartlettii* from Costa Rica and Texas. *Pterocladiella bartlettii* is recorded from Malaysia for the first time in this study. The species was introduced by Taylor (1943) based on the specimens collected from Haiti, Central America (as *Pterocladia bartlettii* Taylor) and transferred to the genus *Pterocladiella* by Santelices (1998). The species was later reported from Texas, North America, Caribbean Islands, South America, Central America and Western Atlantic (Guiry & Guiry 2012; Thomas and Freshwater, 2001; Wynne, 2011). Molecular data of the species in GenBank are from Costa Rica in Central America and Texas (Thomas and Freshwater, 2001). The analyses of our present study showed sister relationship between *P. bartlettii* and the new proposed species, *Pterocladiella* sp. nov.2, from Malaysia and grouped them in a monophyletic clade with *P. melanoidea* from Spain (Figs. 4.16, 4.17, 4.18, 4.23, 4.24 & 4.25).

In phylogenetic analyses of *cox*I gene these specimens were strongly to fully grouped together near the base of the tree as in the *rbc*L phylogenetic tree (Figs. 4.16, 4.17). Very low sequence variation among Malaysian specimens of *P. bartlettii* (0-0.2% in *rbc*L & 0 - 0.7% in *cox*I) and low intraspecific divergence from *P. bartlettii* from Haiti and Texas (0 - 0.3%) (Table 4.8, 4.9) resolved them as the same species. High divergence between *P. bartlettii* from Malaysia, Costa Rica and Texas and

Pterocladiella sp. nov.2 (8.6 - 9% in *rbc*L & 10.9-11.6% in *cox*I) resolved them as two distinct individual species and high divergences between *P. bartlettii* from Malaysia, and *P. melanoidea* (8.4 – 9.5% in *rbc*L) differentiated them as two distinct species.

In LSU gene analyses, also two sequences of *P. bartlettii* from Malaysia resolved in the subclade *P. bartlettii* from Haiti and Texas by strong to full support (Fig. 4.18).

5.2.2 *Pterocladiella beachiae* Freshwater in Thomas & Freshwater

Pterocladiella beachiae was reported for the first time from Costa Rica (Thomas & Freshwater 2001) based on morphological studies and molecular analyses of *rbc*L and LSU genes. The species, however, was suggested as a synonym of *P. caerulescens* based on molecular studies on LSU and SSU sequences which did not show strong support for the species as a distinct taxon (Tronchin and Freshwater, 2007). Extensive collection of Gelidiales from South Africa and Panama in Caribbean sea and *cox*I gene analyses and DNA barcoding, led to reassessment of *P. beachiae* as an individual cryptic species and concluded that the *P. "caerulescens*" complex comprised three individual species including *P. caerulescens* from Hawaii, *P. beachiae* from Costa Rica and Panama and *P. australafricanensis* from South Africa (Freshwater *et al.*, 2010).

Morphological and molecular data both confirmed the presence of *Pterocladiella beachiae* in Malaysia. However some characters such as occasional presence of unbranched erect axis, downward tapering and less constricted base of lateral branches in Malaysian specimens do not agree well with the original description of the *P. beachiae*. Morphological characters of *P. beachiae* such as branches tapering at the base and apex, absence of sterile margin and concave protuberance in matured tetrasporangial sori in *P. beachiae* are the main features which distinguish the species

from *P. caerulescens*. Detailed morphological comparison of *P. beachiae* and *P. caerulescens* (Table 4.1) revealed more differences between these two species. *P. beachiae* had thicker and more compressed stolon; thinner erect axis; shorter, wider and thicker tetrasporangial sori and cystocarps; larger outer and inner cortical cells in erect axis; smaller cortical and inner cells in stolons and thinner rhizoidal attachment system which distinguished it from *P. caerulescens* (Table. 4.1.5).

Phylogenetic analyses of *rbcL* , *cox*I and LSU gene sequences of the specimens collected from different areas in Malaysia moderately to fully resolved them within *P. beachiae* (Figs. 4.16-18) which had been reported from Costa Rica and Panama in Central America (Thomas and Freshwater, 2001). In spite of some variation in habit and morphology of collected specimens, low sequence divergence of Malaysian specimens (0.0 - 0.4% in *rbc*L and 0.7- 0.8% in *cox*I) and also low biogeographical divergence between Malaysian, Caribbean and Central American specimens (0.6 –0.8% in *rbc*L and 2.0 -2.7% in *cox*I, 0.0% in LSU) (Tables 4.8-10) showed they were the same species. Greater interspecific *cox*I variation, at least two times greater than *rbc*L divergence, were reported for intraspecific levels of *Gelidium crinale* and *Gelidium coulteri* (Freshwater *et al.*, 2010). Higher *rbc*L and *cox*I divergence within sequences of *P. beachiae* from the two biogeographic regions reflects biogeographical barriers. The Malaysian specimens of *P. beachiae* and *P. caerulescens* have higher divergence (2.6-3.5% in *rbc*L, 5.1-5.5% in *cox*I & 0.2 % in LSU) than the divergence between *P. beachiae* from of Caribbean Sea and *P. caerulescens* from Hawaiian Island (2.4-2.6% in *rbc*L, 4.5-4.7% in *cox*1 & 0.09% in LSU). This strengthens our observation that *P. caerulescens* and *P. beachiae* from Malaysian are distinct species.

In spite of many records of *P. caerulescens* from the Indo-Pacific region only two *cox*I gene sequences of *P. caerulescens* (HQ412475, HQ412476) (Freshwater et al. 2010) and two sequences for *rbc*L (AF305805 & EF190250) (Thomas and Freshwater, 2001; Tronchin and Freshwater, 2007) have been reported. The sequences of *rbc*L and *cox*I genes provided in this study are the first reports of *P. caerulescens* and *P. beachiae* from Malaysia.

5.2.3 *Pterocladiella caerulescens* (Kützing) Santelices et Hommersand

Pterocladiella caerulescens has been reported from Malaysia based on morphological studies (Silva *et al.*, 1996; Phang *et al.*, 2007). The specimens collected from Malaysia in this study matched well morphologically with the various descriptions and illustrations of *P. caerulescens* (Santelices, 1976, 1977, Santelices and Hommersand, 1997; Santelices, 1978; Xia *et al.*, 2004; Millar and Freshwater, 2005) and were confirmed based on molecular analysis of the *rbc*L and *cox*I gene sequences.

Molecular analyses resolved Malaysian specimens within *P. caerulescens* in the subclade that also contained sequences of *P. caerulescens* from Hawaii based on *rbc*L (Fig. 4.16) and *cox*I genes (Fig. 4.17). This subclade was also grouped with *P. psammophila*, *P. australafricanensis* from South Africa and *P. beachiae* from Panama, Costa Rica and Malaysia, in a complex clade. Interspecific divergence between *P. caerulescens* and *P. beachiae* from Malaysia (2.6-3.5% in *rbc*L and 5.1-5.5% in *cox*I) is more than the divergence between *P. caerulescens* from Hawaii and *P. beachiae* from the Caribbean sea (2.8% in *rbc*L and 4.5-4.7% in *cox*I) which is relatively similar to interspecific divergence between *P. caerulescens* from Malaysia and *P. beachiae* from Caribbean sea (2.3 % in *rbc*L and 4.3-4.5% in *cox*I) (Tables 4.8 & 4.9). These results

showed divergence between *P. caerulescens* and *P. beachiae* is not biogeographic divergence and revealed the fact that the two species are distinct.

Phylogenetic analyses of LSU gene data showed all species of "*P. caerulescens*" complex were grouped in the same subclade (Figs. 4.18 & 4.25). Pairwise sequences divergence of Malaysian *P. caerulescens* from Hawaiian and African specimens of *P. caerulescens* (0.0 – 0.1%) and from Malaysian and Caribbean specimens of *P. beachiae* (0.0-0.3%) showed that LSU is not a good molecular marker for discrimination between closely related species.

5.2.4 *Pterocladiella* sp. nov. 1

Morphological comparison of *Pterocladiella* sp. nov.1 with other species of the Pterocladiacece (Table 4.1) revealed the diagnostic features of this species, to be its minute size; lateral branches originating at right angles to the main axis; obscure main axis in matured thalli; corymbose habit; 2-3 layers of cortical cells in cross section of erect and prostrate axes; and periclinal arrangement of all cortical cell layers in stolons that clearly distinguish it from the closest group including *P. psammophila* and *P. australafricanensis* from Africa (Tronchin and Freshwater, 2007), *P. beachiae* from Central America (Thomas and Freshwater, 2001) and *P. caerulescens* reported from several geographical areas such as the Pacific Islands including Hawaii, the Caribbean, South America, Central America, Africa, Asia, Indian Ocean, South-west Asia, South-east Asia, New Caledonia (Silva *et al*., 1996; Phang *et al*., 2007, Guiry and Guiry 2012).

Molecular data for the genus *Pterocladiella* are only available for 11 of 17 recognized species and varieties. Morphological comparison between *Pterocladiella* sp. nov.1 and the other small species of *Pterocladiella* is shown in Table 4.1. *Pterocladiella* sp. nov.1 is distinguished from *P. minima* (Guiry and Womersley, 1992) Santelices & Hommersand from Australia (Womersley, 1994) and Africa (Lipkin and Silva, 2002) by its right-angled branches and corymbose habit, 1-3 transverse rows of medullary cells in transverse section, and the larger and thicker rhizoids. *P. minima* is uniaxial, with one row of medullary cells (Table 4.1).

P. sanctarum (Feldmann & Hamel) Santelices, another small species of *Pterocladiella*, reported from Central America, South America, Caribbean Islands, Indian Ocean and Western Atlantic (Guiry and Guiry, 2012) is distinguished from *Pterocladiella* sp. nov.1 by its larger size (up to 2 cm), branching pattern, slender form of upper branches, smaller size of tetrasporangial sori, and peg-like rhizoids, as well as size, form and arrangement of cortical cells in transverse section (Table 4.1). *Pterocladiella* sp. nov.1 with its corymbose habit and narrow erect branches (55-240 µm) is distinct from *P. caespitosa* (Kylin) Santelices from Africa, Australia (Guiry and Guiry, 2012; Silva *et al.*, 1996) which has erect branches 1000 µm in width, unbranched or with infrequent distal small branchlets and 4-5 transverse rows of large medullary cells (Santelices, 1998) (Table 4.1). *P. taylorii*, another minute species of *Pterocladiella*, is differentiated from *Pterocladiella* sp. nov.1 by its cylindrical erect and creeping axes, rarely branching, different size and arrangement of cortical cells and 7-8 rows of medullary cells (Santelices, 1998) (Table 4.1).

Based on phylogenetic analyses this species clearly belongs to the Pterocladiaceae in terms of vegetative and reproductive structures. Typical features of *Pterocladiella*

and *Pterocladia* in the family Pterocladiaceae (Perrone *et al*., 2006) include the peg-like rhizoidal attachment consisting of parallel coalescent unicellular filaments which originate from internal cortical cells surrounded by multicellular exogenous filaments, presence of internal rhizines, and the structure of tetrasporangial sori.

Molecular analyses of the *rbc*L and *cox*I gene resolved *Pterocladiella* sp. nov.1 as a distinct monophyletic subclade (Figs. 4.16, 4.17, 4.23, & 4.24). Pairwise divergences of *Pterocladiella* sp. nov.1 from sequences of other species included in this study (5.3-12.3% for *rbc*L and 12.3-20.1% for *cox*I) were much greater than the general divergence values for *rbc*L (≥2%) observed between pairs of congeneric species (Freshwater and Rueness, 1994; Freshwater *et al.,* 1995; Millar and Freshwater, 2005; Freshwater *et al.,* 2010) and also more than *rbc*L divergence (4-5%) and *cox*I divergences (10.1–10.2%) between *Gelidiella acerosa* (Forsskål) Feldmann & Hamel and *Gelidiella fanii* S.-M. Lin and between *Gelidium pristoides* (Turner) Kützing and *Gelidium foliaceum* (Okamura) E.M. Tronchin (Freshwater *et al*., 2010, Lin & Freshwater 2008, Wiriyadamrikul *et al.*, 2010).

5.2.5 *Pterocladiella* sp. nov. 2

The presence of peg-like rhizoidal holdfasts, the V-shaped arrangement of tetrasporangia and the presence of rhizines confirm the position of *Pterocladiella* sp. nov.2 in the family Pterocladiaceae and the genus *Pterocladiella* (Perrone *et al*., 2006).

Morphological comparison with other *Pterocladiella* species (Table 4.1) showed that *Pterocladiella* sp. nov.2, with one to three rows of medullary cells is distinct from *P. taylorii* with 7-8 rows of large medullary cells. Very small size and longer rhizoidal

holdfast with semi-compressed to compressed upper axes, larger tetrasporangial sori, and the anticlinal arrangement of outer cortical cells distinguish *Pterocladiella* sp. nov.2 from *P. sanctarum* which is tubular in the upper axes, has smaller tetrasporangial sori and larger isodiametric outer cortical cells (Table 4.1).

Pterocladiella sp. nov.2 is distinguished from *P. minima* by its smaller size, thinner stolons and erect branches, smaller tetrasporangial sori and more branching (Table 4.1). Larger rhizoids, thinner erect branches and lower number of medullary cell rows in *Pterocladiella* sp. nov.2 distinguish it from *P. caespitosa* (Santelices, 2007).

Pterocladiella sp. nov. 2 showed sister relationship with *Pterocladiella bartlettii* which had monophyly with *P. melanoidea* . They shared the features such as periclinal arrangement of outer cortical cells, the transverse arrangement of large medullary cells, V-shaped tetrasporangial sori and low number of rhizines. However *Pterocladiella* sp. nov.2 is differentiated from these two species that have larger size, blackish colour, long linear lanceolate bilateral or pinnate branches and intercalary large expanded tetrasporangial sori (Dixon and Valera, 1961; Santelices, 1998; Barbara and Tapia, 2012).

All molecular analyses for *rbc*L, *cox*I and genes resolved *Pterocladiella* sp. nov.2 as a distinct clade in the genus *Pterocladiella* with high to full support (Figs. 4.16, 4.17, 4.18, 4.23& 4.24). For LSU only one sequences of *Pterocladiella* sp. nov.2 was available. Sequences of *Pterocladiella* sp. nov.2 from Teluk Kemang and Pulau Pinang showed very low divergence (0 – 0.3% in *rbc*L and 0 – 0.2% in *cox*I) (Table 4.8, 4.9) while divergence between sequences of this species and *P. bartlettii* and *P. melanoidea*, its closest relatives, (8.6–11.3% for *rbc*L and 10.9 -11.6% for *cox*I) indicate that the

species are distinct (Freshwater and Rueness, 1994; Freshwater *et al.*, 1995; Millar & Freshwater, 2005; Freshwater *et al*., 2010). LSU gene phylogenetic analyses showed weak sister relationship (ML=64% in BI=55%) between sequences of *Pterocladiella* sp. nov.2 and *P. melanoidea* from Spain and both are grouped with *P. bartlettii* from Costa Rica and Texas in a monophyletic group by moderate to strong support (ML=71% & BI=90%) (Fig. 4.18).

5.2.6 *Aphanta* sp.

Morphological comparison of Malaysian *Aphanta* sp. with *Aphanta pachyrrhiza* from South Africa (Table 4.2) showed that Malaysian specimens in terms of whole plant size, narrower erect axis, form of erect branches and smaller size of outermost cortical cells, inner cortical cells and larger rhizines, was distinguished from *Aphanta pachyrrhiza* in South Africa.

Molecular analyses of *rbc*L resolved the sequences of Malaysian *Aphanta* sp. within Aphanta *pachyrrhiza* (Fig. 4.16), while LSU analyses showed distinction of Malaysian *Aphanta sp.* from *A. pachyrrhiza*. (Figs. 4.18, 4.25). Although *cox*1 sequences data of *Aphanta pachyrrhiza* species were not available in GenBank, but result of this study showed in *cox*1 gene analyses, the Malaysian specimens of *Aphanta* formed a clade distinct from other members of Pterocladiaceae (Figs. 4.17- 4.23).

Pairwise divergence of these three genes data also showed low divergence among Malaysian specimens sequences (0.0% in *rbc*L, 0.0-0.3% in *cox*1 & 0.0-0.1% in LSU) (Tables. 4.8-10). Divergence of the Malaysian specimens from *Aphanta pachyrrhiza* (9.8-9.9% in *rbc*L & 2.2-2.3% in LSU) (Tables. 4.8 & 4.10) was in the range

representing two distinct species. These ranges of divergence in *rbc*L and LSU genes in order Gelidiales are in the level that represents distinction between two species. Similarly *P. caloglossoides* is shown to be a distinct species from *P. caerulescens* with similar level of divergence (9.2% in *rbc*L & 1.1-1.2% in LSU) (Tables 4.8 & 4.10).

The *cox*1 gene sequences of the Malaysian specimens of *Aphanta* showed high level of divergence from other species of the genus *Pterocladiella* (15.5-19.9%). The divergence between the sequences of Malaysian *Aphanta* and *Aphanta pachyrrhiza*, from *Pterocladia lucida* (14.2-17.3% in *rbc*L & 5.0-6.8% in LSU) is high. *P. lucida* also has high divergence from other species of Pterocladiaceae (13.4-16.1% in *rbc*L, 4.4 -6.1) (Tables 4.8 & 4.10). These showed the *Aphanta* and *Pterocladia lucida* are distant from other species of Pterocladiaceae. This range of divergence represent the level of divergence between two families as seen about *Ptilophora coppejansii* in the family Gelidiaceae from *Gelidiella acerosa* in the family Gelidiellaceae(Tables 4.8 & 4.10), whereby the two are distant families in order Gelidiales. Following this *Pterocladia* and *Aphanta* may be separated into two new families based on the high level of divergence; however *Pterocladia* is the type genus of family Pterocladiaceae.

More morphological and molecular data are needed to provide a robust support for establishment of the new family. The results of this study confirmed the findings of Tronchin and Freshwater (2007) for the establishment of the new genus *Aphanta* based on *rbc*L, SSU and LSU gene analyses.

5.3 FAMILY GELIDICEAE

5.3.1 *Gelidium* cf. *crinale* (Turner) Gaillon var. *perpusillum* Piccone et Grunow

This variety of *Gelidium* was stablished based on population of *Gelidium* grows on the roots of mangroves trees (Dawson, 1954). The variety has reported from Masssawa, Eritrea, Ethiopia in Africa (Silva *et al.*, 1996). The variety has also been reported from Africa (Papenfuss, 1968), Indian Ocean islands (Silva *et al.*, 1996), Indonesia (Silva *et al.*, 1996 & Atmadja and Prud'homme van Reine, 2012), Philippines (Silva *et al.*, 1987; Trono, 1973; Cordero, 1977), Australia and New Zealand (Lewis, 1984, Bostock and Holland, 2010) and Fiji (N'Yeurt *et al.*, 1996).

Morphological features of the species collected from Malaysian coastlines were verified by the illustrated figures of the species in Dawson (1954, page 421). The characteristics are cylindrical thallus, rare branching of the erect axis, apical reproductive structures, large rounded medullary cells with regular arrangement in transverse section and periclinal arrangement of the cortical cells (Table 4.3).

Phylogenetic analyses of *rbc*L and *cox*I sequences showed there is no relationship between the Malaysian specimens and sequences of *G. crinale* from several localities of the world (Figs. 4.19 & 4.20). Many sequences of *rbc*L and *cox*I genes from *G. crinale* were provided and analyzed by Kim *et al.* (2012). Comparison of the sequences of the Malaysian species with sequences of *G. crinale* showed that the Malaysian species is distinct from all sequences of *G. crinale* (Figs. 4.19, 4.20, 4.23 & 4.24).

High pairwise divergence of the Malaysian *Gelidium* sp. from other species of *Gelidium* (Table 4.12) confirmed the separation of the species from other members of the family Gelidiaceae.

We would like to propose a new combination *Gelidium perpusillum* combining the Malaysian *Gelidium* and *G. crinale* var. p*erpusillum* from Africa by elevating the variety to species level. However last confirmation need sequences data from *G. crinale* var. *perpusillum.*

5.3.2 *Gelidium* sp. nov.1

Gelidium sp. nov.1 had some morphological similarity to *G. usmanghanii* Afaq-Husain et Shameel, reported from Pakistan (Afaq-Husain and Shameel, 1996, 1999) especially in branching pattern and reproductive stichidia forms. But the height of *G. usmanghanii* was reported as 7cm, which is five times higher than *Gelidium* sp. nov.1. Width of erect branches and stolon diameter also is much more than *Gelidium* sp.nov.1 (Table 4.3). In rhizine distribution *G. usmanghanii* differed from *Gelidium* sp. nov.1, no rhizine reported among medullary cells of *G. usmanghanii*, while in *Gelidium* sp. nov.1, abundant rhizines were observed among medullary cells although rarely in internal cortex.

In phylogenetic analyses of both *rbc*L and *cox*I genes, the sequences of species formed identical group with full support in all methods of analyses for *rbc*L and strong to full support for *cox*I gene (Figs. 4.19 & 4.20). In *rbc*L gene sequences analyses *Gelidium* sp.nov.1 showed sister relationship with *Gelidium omanenes* from Oman sea by weak to moderate support (Fig. 4.19) and both were grouped in the same clade with

G. pluma from Hawaii, USA with moderate to strong support. In *cox*1analyses, sequences of *Gelidium* sp. nov.1 showed sister relationship with *G. pluma* from Hawaii with moderate to full support (Fig. 4.20).

Sequence divergence of *Gelidium* sp. nov.1 from *G. omanenes* in r*bc*L analyses (5.1-5.2%) showed that the two species are distinct species (Table 4.13). Divergence of *cox*I gene sequences of *Gelidium* sp.nov.1 from *G. pluma* was 9.3-9.7% (Table 4.14), which represents interspecific divergence and showed the Malaysian specimens are distinct from *G. pluma*.

5.3.3 *Gelidium* sp. nov.2

Gelidium sp. nov.2 is grouped in the clade containing *Gelidium divaricatum, G. hommersandii, G. caulacantheum* and *Capreolia implexa* (Figs. 4.19 & 4.20). Morphological comparison with *G. caulacantheum* from Australia and New Zealand the plant of *Gelidium* sp.nov.2 from Malaysia is very different both in whole size and form and also in internal structure and reproductive stichidial branching. *Capreolia* another genus of Gelidiaceae shared the same phylogenetic ancestor with the clade that includes *Gelidium* sp.nov.2. *Gelidium* sp. nov.2 was similar in habit and gross morphology to *Capreolia implexa*, the sole species of the genus *Capreolia*, with some difference in size and internal structure. The main character in establishment of *Capreolia* was biphasic life-cycle (Guiry and Womersley 1993). This feature is not reported for *G. caulacantheum, G. hommersandii* and *G. divaricatum.* In the present study all phylogenetic analyses has confirmed the separation of the clade containing *Capreolia* and three *Gelidium* species, *G. caulacantheum, G. hommersandii* and *G. divaricatum,* in this clade from the other species in the clade of *Gelidium* and clade of *Ptilophora* (Figs.

4.19 & 4.20). There is a need to revise description of the *Capreolia* to accommodate the phylogenetically close species, *G. caulacantheum*, *G. hommersandii* and *G. divaricatum*.

In phylogenetic analyses of *rbc*L gene, four sequences of *Gelidium* sp. nov.2 from Teluk Kemang, and Kuala Terengganu formed a distinct subclade with low to full support in phylogenetic trees and showed sister relationship with *Gelidium divaricatum* Martense from South Korea and Japan by full support in all analyses, and both grouped by strong to full support (Ml=99%, MP=89% & BI=100%) in a monophyletic group with the clade containing *Capreolia simplex*, *Gelidium caulacantheum*, and *Gelidium hommersandii* from New Zealand and Australia (Fig. 4.19).

In *cox*I gene sequence analyses, four sequences of the species formed a distinct clade with moderate to full support and showed sister relationship with *G. divaricatum* with moderate to strong support and both grouped with *G. caulacantheum* with strong to full support (Fig. 4.20).

Pairwise divergence of the species from *G. divaricatum* (3.4-4.4% in *rbc*L and 12.2-12.6% in *cox*I) (Tables 4.12 & 4.13) showed the species is distinct from *G. divaricatum*. The *rbc*L sequences divergence of the species from *G. caulacantheum* (8.1-9.4%), *G. hommersandii* (8.2-9.4%) and from *Capreolia implexa* (8.1-9.1%) shows they are in the range of interspecific level.

Morphologically *Gelidium* sp. nov.2 is similar to the *G. divaricatum* Martens which originally reported from Hong Kong (Martens, 1866) and also has been reported

from China (Tseng, 1984, Santelices, 1988, Zhang & Xia 1988, Xia *et al.,* 2002, Wang, *et al.,* 2003, Xia 2004), Hong Kong (Zhang & Xia 1988), Japan (Okamura 1936; Segawa, 1981; Yoshida *et al.,* 1990; Yoshida, 1998), Korea (Lee, 1994; Lee & Kim 1995; Lee & Kang 2001; Lee 2008), Taiwan (Huang, 2000; Anon,. 2012), Philippines (Silva *et al.,* 1987), Singapore (Pham *et al.,* 2011), Vietnam (Pham-Hoàng, 1969), but size of whole plant and size of erect axis is much smaller than *G. divaricatum* which is 20 mm in height and erect axis of 400-700 μm, that is three times more than the Malaysian specimens (Table 4.3). In internal morphology, Malaysian specimens showed more cortical layers and rounded medullary cells; while *G. divaricatum* from Korea has 3-4 side armed medullary cells (Lee, 1994). Form and size of tetrasporangial stichidia also showed some difference.

5.4 FAMILY GELIDIELLACEAE

5.4.1 *Gelidiella acerosa* (Forsskål) Feldmann & G. Hamel

Sequences of Malaysian *G. acerosa* formed an identical group with strong to full support in the clade *Gelidiella* of the family Gelidiellaceae (Fig. 4.21). The group of Malaysian sequences were resolved into Caribbean and east African subclade with weak to strong support and showed sister relationship with the species of Caribbean by weak to strong support, while all Pacific specimens of *Gelidiella acerosa* including specimens of Australia, Hawaii, New Caledonia, Philippines and Taiwan formed the other subclade in the clade *Gelidiella.*

Pairwise divergence of *rbc*L gene sequences of Malaysian specimens of *G. acerosa* from Caribbean and Eastern African specimens (1.1-1.5%) was lower than their

divergence from Australian, Hawaiian and New Caledonian specimens (2.3-3.3%) and from Taiwan and Philippines (2.8-4.6%) (Table 4.15).

Analyses of *cox*I gene also resolved the sequences of Malaysian specimen within the clade *Gelidiella acerosa* with the sequences of species from Philippines by strong to full support, while species of *Gelidiella acerosa* from Hawaii, reported by Sherwood et al (2010) grouped with the *Gelidiella fanii* from Philippines, Thailand and Indonesia by full support for all methods of analyses (Fig. 4.22).

Pairwise divergence of *cox*I gene sequences of Malaysian *G. acerosa* (0.3%) was within the range representing the same species (Freshwater *et al.,* 2010) and their divergence from the sequences of Philippines (0.2-0.5%) showed they are also within the range of same species. *Cox*I sequence divergence of Malaysian *G. acerosa* from Hawaiian sequences (11.9-12.5%) was similar to the divergence of Malaysian specimens from *Gelidiella fanii* reported from Thailand, Philippines and Indonesia, which was similar to the divergence of *G. acerosa* from *G. fanii* (11.2-12.1%).

There is no morphological data for *G. acerosa* from Costa Rica and Puerto Rico, only their *rbc*L sequences had been deposited in the GenBank by Thomas & Freshwater (2000) and from Tanzania by Lin & Freshwater (2007). The Malaysian species of *G. acerosa* had most of the features described for the species of *G. acerosa* from Taiwan (Lin & Freshwater, 2008) with some difference in branching pattern in Malaysian specimens, showing both unilateral branching in first order and alternate to subopposite branching in second order (Fig.4.10A). *Gelidiella acerosa* have been described as having basal hair cells among cortical cells (Lawson & John, 1987; De Clerck *et al.,* 2005; Taylor, 1960; Littler & Littler, 2000; Norris, 1992; Woelkerling, 1976). Presence

of brownish basal hair cells were the characters which were not observed in the Taiwan species but Malaysian species showed this character among cortical cells of erect branches (Fig.4.10D). Compressed ligulate branches at basal portions of erect axes (Fig.4.10A) was another feature which had not reported for Taiwan species. Size of branches and erect axes were measured wider than Taiwan species.

5.4.2 *Parviphycus* sp.1

In phylogenetic analyses three sequences of Malaysian *Parviphycus* sp.1 in both *rbc*L and *cox*I genes analyses formed a distinct group (Figs. 4.21 & 4.22). In *rbc*L analyses sequences of *Parviphycus* sp. 1 grouped together by strong to full support (ML= 85%, MP=83% & BI=100%). This subclade has shown relationship to another three sequences of *Parviphycus* from Malaysia and all showed sister relationship to *P. antipai* from Australia with full support (Fig. 4.21). Pairwise sequence divergence of of the *Parviphycus* sp.1 (0.0-0.1%) (Table 4.15) and their similarity in morphology showed these specimens belong to same species.

Two specimens of *Parviphycus* sp.2 are morphologically very different from *Parviphycus* sp.1 but showed low divergence with *Parviphycus* sp. 2, (0.1-0.2%) and *Parviphycus* sp. 3 (0.9-1.0%) that is morphologically different but placed in the same subclade with *Parviphycus* sp.1 and *Parviphycus* sp.2. This makes it difficult to make any decision on the species separation.

The genus *Parviphycus* was introduced by Santelices (2004) based on the morphological differences of minute species of *Gelidiella* with *Gelidiella acerosa*, the type species of the family Gelidiellaceae. Features such as distichous division of subapical cell, regular type (Pannosa-type) of tetrasporangial stichidia and internal

thallus structure with transverse row of axial and periaxial cells and position of tetrasporangia in transverse section were the characteristics used for separation of this group of minute *Gelidiella* and accommodating them in the new genus *Parviphycus*. Consequently three typical species of this group including *Gelidiella adnatus* Dawson, *G. antipai* Celan, *G. tenuissimus* Feldman et Hamel were transferred to the genus *Parviphycus. Gelidiella trinitatensis* W. R. Taylor, another minute species of the *Gelidiella* were transferred to the genus *Parviphycus* by Wynne (2011).

This genus was confirmed by molecular studies based on *rbc*L and LSU gene sequences (Rico *et al.*, 2002; Millar and Freshwater 2005; Huisman *et al*., 2009).

The present study showed some of the minute species of Gelidiellaceae from Malaysia phylogenetically were grouped in clade *Parviphycus*, The Malaysian specimens were distantly placed from *P. antipai* and *P. pannosus* (the molecular sequences in GenBank recorded as *P. tenuissimus*, but based on the priority article the name were suggested as synonym for *P. pannosus* by Furnari *et al.*, 2010). Masuda *et al.* (2000) reported *Gelidiella pannosa* from Pulau Nyior, Pulau Pasir and Pulau Langkawi in Malaysia. All the morphological features reported for the Malaysian *Gelidiella pannosa* are well matched with the description of *Parviphycus* sp.1. This shows the *Gelidiella pannosa* (=*Parviphycus pannosus*) reported from Malaysia should not be *P. pannosus*.

Three *cox*I gene sequences from *Parviphycus* sp.1 reperesent first sequences of this genus formed a new clade in the *cox*I gene phylogenetic tree in Gelidiellaceae. Low divergence among this group also put them in same taxon (Fig.4.21 & 4.22).

5.4.3 *Parviphycus* sp. 2

Two sequences of *rbc*L genes of *Parviphycus* sp.2 grouped with *Parviphycus* sp.1 specimens of Malaysia with a sister relationship to *P. antipai*. The divergence of these two sequences from *Parviphycus* sp.1 was low (0.1-0.2%) and from *P. antipai* was 7.2%.

Morphologically the specimens *Parviphycus* sp.2 are distinct from of *P. antipai, P. adnata, P. pannosus,* because of its large size, extensively branched and entangled axes, position and form of tetrasporangial stichidia with stellate to moniliform aggregation of tetrasporangial stichidia, medulla cells size and arrangement.

The morphologicaly closest taxon to *Parviphycus* sp.2 is *P. trinitatensis* . Based on description of *P. trinitatensis* (Taylor, 1943, Wynne 2011) the pinnate and palmate branching forms of tetrasporangial stichidia showed some similarity to *Parviphycus* sp.2 (Table 4.4), but the minute size of *P. trinitatensis*, entire erect axes or rarely sparingly branching, lower axes diameter, are different with *Parviphycus* sp.2.

5.4.4 *Parviphycus* sp.3

Parviphycus sp.3 from Malaysia is similar to *Gelidiella ramellosa* especially in the form of tetrasporangial stichidia but vegetative structures especially branching pattern are distinct from *G. ramellosa*. In vegetative structure, plants of the specimens showed some similarity with *Gelidiella myrioclada* (Børgesen) Feldman & Hamel and was similar to the illustrated figures by Dawson (1954, page 423) but the tetrasporangial stichidia are different. Therefore more study is required to clarify identity of the species.

Parviphycus sp.3 was grouped with *Parviphycus* sp.1 and *Parviphycus* sp.2 and all showed sister relationship with *P. antipai* with 7.2% *rbc*L sequences divergence, which showed that it is distinct from *P. antipai*. Morphologically, specimens of the plants are similar to *Gelidiella ramellosa* especially in forms of tetrasporangial stichidia, but phylogenetically *Parviphycus* sp.3 did not show close relationship with *G. ramellosa* and sequence divergence between two species was high (11.8-12.0% in *rbc*L).

This study has provided many new sequences data of Gelidiales collected for the first time from Malaysia and has increased the number of *Malaysia* Gelidiales species to 17 distinct species, of which four are proposed as new species for the world, two are new record for Malaysia and Indo-Pacific region and three taxa show potential as new species but their final identify need more study by other genetic markers.

The molecular analyses of this study verified the previously reported species of *Pterocladiella caerulescens* and *Gelidiella acerosa* (Phang, 2006; Phang *et al.*, 2007; Silva *et al.*, 1996) and provided detailed morphological description for these taxa growing in Malaysian.

5.5 Interfamilial Relationship of order Gelidiales

Phylogenetic analyses of the order Gelidiales based on partial sequences of three genes (*rbc*L, *cox*I & LSU) acquired from the GenBank and the sequences obtained from this study provided good data for overall phylogenetic evaluation of the order.

Phylogenetic analyses of the order based on 151 sequences of *rbc*L gene including 44 sequences of Malaysian specimens obtained in this study showed that Gelidiaceae has the most sequences of the order Gelidiales in the GenBank. These analyses showed Gelidiaceae in not a monophyletic family, as two clades *Capreolia* and *Ptilophora* did not show monophyly with the clade *Gelidium*, which contains the type species. The clade *Capreolia* is a heterogenetic clade containing *Capreolia implexa*, *Gelidium caulacantheum*, *G. hommersandii*, *G. divaricatum* and *Gelidium sp.nov*.2, the newly proposed species in this study. All the species of this clade are derived from the same ancestor as had been reported in previous studies (Freshwater and Rueness, 1994; Freshwater *et al.*, 1995; Bailey and Freshwater, 1997; Freshwater and Bailey, 1998; Millar and Freshwater 2005; Nelson *et al.*, 2006, Tronchin and Freshwater, 2002, 2007).

The clade *Ptilophora* does not show strong support for monophyly with *Gelidium* clade and *Capreolia*, although seemingly all species of the clade belong to the genus *Ptilophora*, as shown in the morphological studies by Norris (1990, 1992) and molecular analyses by Tronchin *et al.* (2003, 2004) who merged the species of genus *Beckerella* with *Ptilophora.*

General analyses of *rbc*L sequences of Gelidiales showed Pterocladiaceae also is not a monophyletic family (Fig 4.23). This paraphyly has been reported by other studies (Freshwater and Rueness, 1994; Freshwater *et al.*, 1995; Bailey and Freshwater, 1997; Freshwater and Bailey, 1998; Millar and Freshwater, 2005; Nelson *et al.*, 2006, Tronchin and Freshwater, 2002, 2007).

Aphanta, the recently proposed genus in the order Gelidiales (Tronchin and Freshwater, 2007), was another genus with uncertain position in the family

Pterocladiaceae. Finding this genus for the first time in Malaysian and comprehensive analyses of the *rbc*L, coxI and LSU sequences of this genus with most of the available sequences of the order in Genbank showed that the taxonomic position of this genus also needs further clarification. Our study showed that *Aphanta pachyrrhiza* from South Africa and *Aphanta* sp. from Malaysia formed a distinct clade with basal position in *rbc*L, coxI and LSU phylogenetic trees of the Gelidiales (Fig. 4.16, 4.17, 4.18 & 4.23-24-25). Interestingly *rbc*L gene analyses showed this clade was separated from Pterocladiaceae by the clade Gelidiellaceae, as well as the genus *Pterocladia*, which showed closer relationship with Gelidiellaceae than Pterocladiceae. High distant of *Pterocladia lucida* from *Pterocladiella capillacea* and *P. melanoidea* and its closer relationship with *Gelidiella acero*sa has been reported based on the *rbc*L and SSU genes data analyses (Bailey and Freshwater, 1997; Freshwater *et al.*, 1995).

Specimens of the species *Aphanta* sp. were collected from Port Dickson in west coast of Peninsular Malaysia and Puala Munakun, Kota Kinabulu, Sabah in East Malaysia, showing its distribution over a large area of the South China Sea region. The species is much smaller than *A. pachyrrhiza* reported from South Africa, and its axes branching pattern is very different and it also has much branched rhizoidal attachment system, making them morphologically distinct.

The latest classification in the order Gelidiales was proposed by Perrone et al. (2006), who suggested the characterstics of rhizoidal attachment system as the main vegetative characteristic for classification of the families in the Gelidiales. They proposed peg-like attachment system as characteristics of the family Pterocladiaceae, brush-like attachment system for family Gelidiaceae and unicellular independent rhizoidal attachment for family Gelidiellaceae. These characteristics have already been

verified by molecular studies on *rbc*L, SSU and ITS genes data analyses by Shimada *et al.* (2000). In the original description of genus *Aphanta* (Tronchin and Freshwater 2007) in Figure 5, they showed the fibrous form of the stolon, although they did not pay attention to this character as the most characteristics of the genus, however in Malaysian specimens this feature may be diagnostic feature *Aphanta* (Figs.4.6) and separate *Aphanta* from the rest of Gelidiales. This shows the need to elevate this group into a new family in the order.

High *rbc*L divergence of *Aphanta* from the main group of the family Pterocaldiaceae (12.4%), Gelidiaceae (13.1%) and Gelidiellaceae (14.6%) (Table 4.18), high *cox*I divergence (15.9% from Pterocladiaceae, 16.6 % from Gelidiaceae & 17.6% from Gelidiellaceae) (Tables 4.19) and also high LSU divergence (3.8% from Pterocladiaceae, 3.9% from Gelidiaceae & 5.2% from Gelidiellaceae) (Table 4.20) all support this suggestion.

The family Gelidiellaceae is another family of order Gelidiales characterized by absence of rhizine in the *Gelidiella* species that was established by Fan (1961). In the *rbc*L gene analyses of the 151 sequences of the order, paraphyly in the species of the family also was clarified. Analyses of the *rbc*L data from three new sequences of *G. acerosa* and six sequences of *Parviphycus* from Malaysia showed that *Parviphycus* and *Gelidiella* are distant but originated from the same ancestor (Fig. 4.23) but their pairwise sequences divergence (11.7% in *rbc*L, 17.7% in *cox*I & 4.1% in LSU) is larger than the divergence of *Gelidium* spp. from Pterocladiaceae (10.9% in *rbc*L, 17.2% in *cox*I & 4.8% in LSU) (Table s 4.18, 4.19 & 4.20) which indicated the need for more study about this group of Gelidiales.

In this study we found that combination of detailed morphological studies and molecular phylogeny is necessary to identify the Gelidiales in any taxonomic level with high accuracy. Morphological data in this investigation based on anatomical characters of vegetative and reproductive structures of Gelidiales species are able to reflect the phylogenetic relationship at generic and specific level.

This study on Malaysian Gelidiales showed there are potentially more species within the order than previously reported and demonstrated that the H_0 hypothesis can be rejected and also showed the need for more collection and further detailed studies study to determine comprehensive gelidioid seaweed taxonomy in Malaysia.

6.0 CONCLUSION

Previous studies on the marine algal resources of Malaysia reported 396 species belonging to 56 families (Phang *et al.,* 2007), of which only eight species belonging the Gelidiales making up about 2% of Malaysian species.

In this study from 13 morphological groups of specimens collected from ten localities in Malaysian, 11 species were verified by molecular analyses on three genetic markers *rbc*L, *cox*I and LSU. Of these, only two, *Gelidiella acerosa* and *Pterocladiella caerulescens* had been already reported in Malaysia.

Two new species of family Pterocladiaceae are reported. *Pterocladiella* sp. nov.1 formed a new subclade in the Pterocladiaceae based on *rbc*L and *cox*I sequences. In spite of its small size, the species is commonly found on the wave exposed rocks and sand covered boulders and stones at the upper fringe of the middle intertidal regions.

Pterocladiella sp. nov.2, a minute species growing among the basal parts of the larger red algae such as *P. beachiae* and *P. caerulescens* populations at lower parts of intertidal zones on coral reefs. In spite of having the same ecological niche, phylogenetically, this species showed high genetic distance from *P. beachiae* and *P. caerulescens* but was more related to the *P. bartlettii* from Caribbean Sea. The vegetative characteristics which are synapomorphic characters are their V- shaped

tetrasporangial stichidia, periclinal arrangement of cortical cells in transverse section of erect and creeping axes and presence of discoid holdfast at the end of the peg-like rhizoids.

P. bartlettii from Malaysia was phylogenetically resolved into the two sequences of *P. bartlettii* from Haiti in Caribbean Sea and Texas. All three genes, *rbc*L, *cox*I and LSU showed the same phylogenetic position for the species from Malaysian. These results showed the taxonomic value of tetrasporangial stichidia and attachment system features for the taxonomic evaluation of this species. In Gelidiaceae there is potentially two new species of *Gelidium* while the third is compared to *Gelidium crinale* var. *perpusillum*. We also proposed that *Aphanta* be elevated to a new family due to its distance from all other members of Gelidiales.

In general this study showed that molecular data combined with detailed morphological study can solve many taxonomic problems in the order Gelidiales at family, generic, specific and even in intraspecific levels. Two genes *rbc*L and *cox*I are suitable markers for taxonomic evaluation in the generic, specific and intraspecific levels while LSU is suitable marker for generic and family levels.

This study using modern molecular study combined with morphological study has increased the number of species of Gelidiales in Malaysian to 17 and the hypothesis H_0 was rejected.

6.2 Areas for Future Research

The present study showed that more research using combination of molecular and morphological data needs to be done for the Malaysian Gelidiales. The paraphyletic groups such as *Pterocladia*, *Ptilophora*, *Capreolia* and *Parviphycus* in the order requires further investigation. More collections of the above mentioned groups in addition to more morphological and molecular works on type specimens or specimens from type locality are needed to solve the taxonomic problems in this order. Many minute species of the order still remain unknown and there is a more collection and molecular and morphological studies to determine the comprehensive gelidian resources of Malaysia.

6.3 Appraisal of study

With minute sized species it was difficult to clean and sort the same species for DNA extraction. This was especially difficult when number of sample was low. This resulted in insufficient material for getting sufficient pure genomic DNA. Conclusions on some taxa would not be made because sequences for some genes (e.g. *cox*I) are not available in the GenBank for compare with our results. Also it was not possible obtain sequences of type species or even collect species from type localities.

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8.0 APPENDICES

Appendix 1: *Bekerella* species synonymised with species of *Ptilophora*

Species name	**Synonym**
Beckerella biserrata (Børgesen) K.-C.Fan & Papenfuss	
Ptilophora hildebrandtii (Hauck) R.E.Norris	*Beckerella hildebrandtii* (Hauck) Kylin
Ptilophora irregularis (Akatsuka & Masaki) R.E.Norris	*Beckerella irregularis* Akatsuka & Masaki
Ptilophora mediterranea (H.Huvé) R.E.Norris	*Beckerella mediterranea* H.Huvé
Ptilophora pectinata (A.Gepp & E.S.Gepp) R.E.Norris	*Beckerella pectinata* (A.Gepp & E.S.Gepp) K.C.Fan & Papenfuss
Ptilophora pectinata (A.Gepp & E.S.Gepp) R.E.Norris	*Beckerella helenae* (Dickinson) K.-C.Fan & Papenfuss
Ptilophora pinnatifida J.Agardh	*Beckerella pinnatifida* (J.Agardh) Kylin
Ptilophora pinnatifida J.Agardh	*Beckerella beckeri (Holmes) Kylin*
Ptilophora pterocladioides Andriamampandry	*Beckerella pterocladioides* (Andriamampandry) P.C.Silva
Ptilophora rumpii (Dickinson) R.E.Norris	*Beckerella rumpii* (Dickinson) Papenfuss & Fan
Ptilophora scalaramosa (Kraft) R.E.Norris	*Beckerella scalaramosa* Kraft
Ptilophora subcostata (Okamura) R.E.Norris	*Beckerella subcostata* (Okamura) Kylin

Appendix2: List of *Gelidium* species and varieties

Gelidium abbottiorum R.E.Norris	G. concinnum Baardseth	*G. latifolium* var. *luxurians* (Crouan & Crouan) Feldmann & Hamel
G. aculeatum (Greville) Batters	*G. congestum* W.R.Taylor	*G.latiusculum* Okamura
G. aculeatum var. *abnorme* (Greville) Batters	*G. coreanum* Kim, Hwang,Yoon & Boo	*G. lingulatum* Kützing
G. affine Schiffner	*G. corneum* var. *caespitosum* (Turner) J.Agardh	*G. linoides* Kützing
G. allanii V.J.Chapman	*G.corneum* var. *pinnatum* (Hudson) Turner	*G. longipes* J.Agardh
G. amamiense Tanaka & K.Nozawa	*G. corneum* var. *pectinatum* Ardissone & Strafforello	*G. madagascariense* Andriamampandry
G. amansii (J.V.Lamouroux) J.V.Lamouroux	*G. corneum* var. *lepadicola* Postels & Ruprecht	*G. elegans* Kützing
G. amansii f. *teretiusculum* Okamura	*G. corneum* var. *aculeatum* Greville	*G. elminense* Dickinson
G. amansii f. *elatum* Okamura	*G. corneum* var. *simplex* Postels & Ruprecht	*G. eucorneum* Kim, Hwang, Park & Boo
G. amansii f. *latius* Okamura	*G. corneum* var. *subrigidum* Grunow	*G. fasciculatum* G.Hamel
G. ambiguum Piccone & Grunow	*G. corneum* (Hudson) J.V.Lamouroux – type	*G. filicinum* Bory de Saint-Vincent
G.amboniense Hatta & Prud'homme van Reine	*G corneum* var. *abnorme* Greville	*G. flaccidum* P.J.L.Dangeard
G. americanum (W.R.Taylor) Santelices	*G. corneum* var. *setaceum* Kützing	*G. floridanum* W.R.Taylor
G. anthonini J.V.Lamouroux	*G. corneum* var. *claviger* Greville	*G. foliaceum* (Okamura) E.M.Tronchin
G.applanatum Stegenga, Bolton & R.J.Anderson	*G. corneum* var. *confertum* Greville	*G. foliosum* P.J.L.Dangeard
G. arborescens N.L.Gardner	*G. corneum* var. *laciniatum* Kützing	*G. galapagense* W.R.Taylor
G. arbusculum Bory de Saint-Vincent ex Børgesen	*G. corneum* var. *flexuosum* Harvey	*G. hancockii* W.R.Taylor
G. arenarium Kylin	*G. coronadense* E.Y.Dawson	*G. heterocladum* Papenfuss
G. asperum (C.Agardh) Greville	*G. corrigerum* J.V.Lamouroux	*G. hildenbrandtii* (Hauck) F.Schmitz
G. attenuatum var. *confertum* (Greville) Batters	*G. coulteri* Harvey	*G. hommmersandii* Millar & Freshwater
G. attenuatum (Turner) Thuret	*G. crinale* var. *corymbosum* (Kützing) Feldmann & Hamel	*G. howei* Acleto
G. australe J.Agardh	*G. crinale* f. *latifolium* Okamura	*G. hypnosum* Zanardini ex R.Molinier
G. bernabei A.J.K.Millar & D.W.Freshwater	*G. crinale* (Hare ex Turner) Gaillon	*G. hystrix* Zanardini
G. bipectinatum Furnari	*G. crinale* var. *polycladum* (Kützing) Hauck	*G. inagakii* Yoshida
G. canariense (Grunow) Seoane Camba et al.	*G. crinale* var. *luxurians* Collins	*G. indonesianum* Kim, Gerung & Boo
G. cantabricum Seoane-Camba	*G. crinale* var. *platycladum* W.R.Taylor	*G. inflexum* Baardseth
G. capense (S.G.Gmelin) P.C.Silva	*G. crinale* var. *perpusillum* Piccone & Grunow	*G.intertextum* P.Dangeard
G. cartilagineum var. *setaceum* (C.Agardh) Grunow	*G. crispum* M.A.Howe	*G. isabelae* W.R.Taylor
G. caulacantheum J.Agardh	*G. deciduum* E.Y.Dawson	*G. japonicum* (Harvey) Okamura
G. ceramoides Levring	*G. declerckii* Tronchin	*G. jejuensis* Kim, Hwang, Yoon & Boo
G. chilense (Montagne) Santelices & Montalva	*G. decompositum* Setchell & Gardner	*G. johnstonii* Setchell & Gardner

G. clavatum (J.V.Lamouroux) J.V.Lamouroux	*G. delicatulum* (Kützing) Setchell	*G. kintaroi* Yamada
G. claviferum Kützing	*G. divaricatum* G.Martens	*G. koshikianum* Shimada, Horiguchi & Masuda
G. coarctatum Kützing	*G. congestum* W.R.Taylor	*G. maggsiae* Rico & Guiry

Appendix2: List of *Gelidium* species and varieties (Continue)

G. maidenii A.H.S.Lucas	*G. pulchellum* (Turner) Kützing	*G. semipinnatum* Piccone & Grunow
G. masudae B.M.Xia & C.K.Tseng	*G. prostratum* Kim ,Hwang, Yoon & Boo	*G. senegalense* Feldmann
G. mcnabbianum (E.Y.Dawson) B.Santelices	*G. pulchellum* var*. claviger* (Greville) Batters	*G. serpens* J.Agardh
G. microdentatum E.Y.Dawson	*G. pulchellum* var. *supradecompositum* (Küzting) Dangeard	*G. serrulatum* J.Agardh
G. microdon Kützing	*G. pulchrum* N.L.Gardner	*G. sinicola* N.L.Gardner
G. microdonticum W.R.Taylor	*G. pulvinatum* f. *parvissimum* Børgesen	*G. sornorense* E.Y.Dawson
G. microphyllum (Crosby Smith) Kylin	*G. pulvinatum* (Kützing) Thuret ex Bornet	*G. spathulatum* (Kützing) Bornet
G. microphysa Setchell & N.L.Gardner	*G. purpurascens* N.L.Gardner	*G. spinosum* var. *hystrix* (J.Agardh) Furnari
G. micropterum Kützing	*G. pusillum* var. *simplex* P.J.L.Dangeard	*G. spinosum* f. *elongatum* (Hatta & Prud'homme van Reine)Silva
G. minimum Kim, Hwang, Yoon & Boo	*G. pusillum* var. *mucronatum* P.J.L.Dangeard	*G. spinosum* (S.G.Gmelin) P.C.Silva
G. minusculum (Weber-van Bosse) R.E.Norris	*G. pusillum* f. *pakistanciu*m Afaq-Husain & Shameel	*G. subfastigiatum* Okamura
G. multifidum Greville C	*G. pusillum* var. *cylindricum* W.R.Taylor	*G. tenue* Okamura
G. musciforme (W.R.Taylor) Santelices	*G. pusillum* (Stackhouse) Le Jolis	*G. tenuifolium* Shimada, Horiguchi & Masuda
G. nova-granatense W.R.Taylor	*G. pusillum* var. pacificum W.R.Taylor	*G. torulosum* Kützing
G. nudifrons N.L.Gardner	*G. pusillum* var. pulvinatum (C.Agardh) Feldmann	*G. tsengii* K.-C.Fan
G. obtusum Schousboe	*G. pygmaeum* (Lightfoot) Lyngbye	*G. umbricola* E.Y.Dawson & Neushul
G. omanense M.J.Wynne	*G. reediae* N.H.Loomis	*G. undulatum* N.H.Loomis
G. pacificum Okamura	*G. refugiensis* (E.Y.Dawson) Santelices	*G. usmanghanii* Afaq-Husain & M.Shameel
G. pannosum f. *exiguum* Weber-van Bosse	*G. regulare* Baardseth	*G. vagum* Okamura
G. parvulum Greville	*G. reptans* (Suhr) Kylin	*G. venetum* Schiffner
G. planiusculum Okamura	*G. rex* Santelices & I.A.Abbott	*G. venturianum* E.Y.Dawson
G. pluma Bornet ex N.H.Loomis	*G. rigens* (C.Agardh) Greville ex Kützing	*G. versicolor* (S.G.Gmelin) J.V.Lamouroux
G. pristoides (Turner) Kützing	*G. rigidum* var. radicans (Bory) J.Agardh	*G. vietnamense* Pham-Hoàng Hô
G. profundum Tronchin & Freshwater	*G. robustum* (N.L.Gardner) Hollenberg & I.A.Abbott	*G. vittatum* (Linnaeus) Kützing
G. proliferum Kützing	*G. rugosulum* Schousboe	*G. vittatum* f. *laceratum* (Grunow) D.W.Freshwater
G. prostratum Kim ,Hwang, Yoon & Boo	*G. samoense* Reinbold	*G. yamadae* K.-C.Fan
G. pseudointricatum Skottsberg & Levring	*G. sclerophyllum* W.R.Taylor	*G. zollingeri* Sonder

G. pteridifolium Norris, Hommersand & Fredericq	*G. secundatum* Zanardini ex Kützing	

Appendix 3: *Gelidium* species synonymised with species of *Pterocladiella*.

Species name	**Synonym**
Pterocladiella caloglossoides (M.A. Howe) Santelices	*Gelidium caloglossoides* M.A. Howe
Pterocladiella melanoidea var. gracilis (Feldmann & G.Hamel) M.J. Wynne	*Gelidium caerulescens* Kützing
Pterocladiella caespitosa (Kylin) Santelices	*Gelidium caespitosum* Kylin
Pterocladiella caerulescens (Kützing) Santelices & Hommersand	*Gelidium irregulare* N.H. Loomis
Pterocladiella tenuis (Okamura) Shimada, Horiguchi & Masuda	*Gelidium decumbensum* Okamura
Pterocladiella capillacea (S.G. Gmelin) Santelices & Hommersand	*Gelidium capillaceum* (S.G. Gmelin) Meneghini
Pterocladiella capillacea (S.G. Gmelin) Santelices & Hommersand	*Gelidium pusillum var. conchicolum* Piccone & Grunow
Pterocladiella caerulescens (Kützing) Santelices & Hommersand	*Gelidium pyramidale* N.L. Gardner
Pterocladiella capillacea (S.G. Gmelin) Santelices & Hommersand	*Gelidium capillaceum* (S.G. Gmelin) Kützing
Pterocladiella caerulescens (Kützing) Santelices & Hommersand	*Gelidium melanoideum* Schousboe ex Bornet
Pterocladiella melanoidea var. filamentosa (Schousboe ex Bornet) M.J. Wynne	*Gelidium melanoideum var. filamentosum* Schousboe ex Bornet
Pterocladiella melanoidea (Schousboe ex Bornet) Santelices & Hommersand	*Gelidium tropicum* (E.Y. Dawson) Verheij & Prud'homme van Reine
Pterocladiella capillacea (S.G. Gmelin) Santelices & Hommersand	*Gelidium melanoideum* Schousboe ex Bornet
Pterocladiella caerulescens (Kützing) Santelices & Hommersand	*Gelidium melanoideum var. filamentosum* Schousboe ex Bornet
Pterocladiella caloglossoides (M.A. Howe) Santelices	*Gelidium tropicum* (E.Y. Dawson) Verheij & Prud'homme van Reine
Pterocladiella melanoidea var. gracilis (Feldmann & G.Hamel) M.J. Wynne	*Gelidium melanoideum* Schousboe ex Bornet

Appendix 4: *Gelidium* species synonymised with other species of *Gelidium.*

Species name	**Synonym**
Gelidium canariense (Grunow) Seoane Camba ex Haroun, Gil-Rodríguez, Díaz de Castro & Prud'homme van Reine	*Gelidium cartilagineum var. canariense* Grunow
Gelidium robustum (N.L. Gardner) Hollenberg & I.A. Abbott	*Gelidium cartilagineum var. robustum* N.L. Gardner
Gelidium kintaroi (Okamura) Yamada	*Gelidium clavatum* Okamura
Gelidium spinosum var. hystrix (J. Agardh) Furnari	*Gelidium contortum* N.H. Loomis
Gelidium purpurascens N.L. Gardner	*Gelidium corneum var. hystrix* J. Agardh
Gelidium crinale (Hare ex Turner) Gaillon	*Gelidium corneum* var. latifolium Greville
Gelidium spinosum (S.G. Gmelin) P.C. Silva	*Gelidium crinale var. spathulatum* (Kützing) Schiffner
Gelidium spathulatum (Kützing) Bornet	*Gelidium corneum* var. crinale (Turner) Greville
Gelidium spathulatum (Kützing) Bornet	*Gelidium crinale var. spathulatum* (Kützing) Hauck
Gelidium yamadae K.-C. Fan	*Gelidium densum* N.L. Gardner
Gelidium purpurascens N.L. Gardner	*Gelidium densum* Okamura
Gelidium purpurascens N.L. Gardner	*Gelidium dictichum* N.H. Loomis
Gelidium purpurascens N.L. Gardner	*Gelidium gardneri* N.H. Loomis
Gelidium asperum (C. Agardh) Greville	*Gelidium glandulaefolium*
Gelidium vagum Okamura	*Gelidium grubbae* Fan
Gelidium spinosum (S.G. Gmelin) P.C. Silva	*Gelidium latifolium* Bornet ex Hauck
*Gelidium spinosum var. hystrix (*J. Agardh) Furnari	*Gelidium latifolium* var. *hystrix* (J. Agardh) Hauck
Gelidium spinosum f. elongatum (Hatta & Prud'homme van Reine) P. Silva	*Gelidium latifolium f. elongatum* Hatta & Prud'homme van Reine

Appendix 4:*Gelidium* species synonymised with other species of *Gelidium* (Continue)

Species name	**Synonym**
Gelidium inagakii Yoshida	*Gelidium nanum* Inagaki
Gelidium minusculum (Weber-van Bosse) R.E.Norris	*Gelidium pusillum* var. *minusculum* Weber
Gelidium purpurascens N.L. Gardner	*Gelidium pannosum* Bornet ex Weber
Gelidium purpurascens N.L. Gardner	*Gelidium papenfussii* N.H. Loomis
Gelidium lingulatum Kützing	*Gelidium lingulatum* J. Agardh
Gelidium corneum var. pectinatum Ardissone & Strafforello	*Gelidium pectinatum* Schousboe ex Montagne
Gelidium spathulatum (Kützing) Bornet	*Gelidium polycladum* Kützing
Gelidium purpurascens N.L. Gardner	*Gelidium polystichum* Gardner
Gelidium pulchellum (Turner) Kützing	*Gelidium pulchellum var. claviferum* (Turner) Feldmann & Hamel
Gelidium pusillum var. pulvinatum (C. Agardh) Feldmann	*Gelidium pulvinatum* (C. Agardh) Grunow
Gelidium foliaceum (Okamura) E.M. Tronchin	*Gelidium supradecompositum* Kützing
Gelidium purpurascens N.L. Gardner	*Gelidium ramuliferum* Gardner
Gelidium pusillum (Stackhouse) Le Jolis	*Gelidium repens* Okamura
*Gelidium corneu*m (Hudson) J.V. Lamouroux	*Gelidium sesquipedale* (Clemente)
Gelidium purpurascens N.L. Gardner	*Gelidium setchellii* N.L. Gardner
Gelidium pulchellum var. supradecompositum (Küzting) P.J.L. Dangeard	*Gelidium pusillum* f. *foliaceum* Okamura
Gelidium inagakii Yoshida	*Gelidium nanum* Inagaki
Gelidium purpurascens N.L. Gardner	*Gelidium pannosum* Bornet ex Weber

Appendix 5: *Gelidium* species have been transferred to the other order of red algae.

Species name	**Synonym**
Tichocarpus crinitus (S.G. Gmelin) Ruprecht	*Gelidium serratum* Kützing
Prionitis decipiens (Montagne) J. Agardh	*Gelidium scoparium* Montagne & Millardet
Trematocarpus flabellatus (J. Agardh) De Toni	*Gelidium rostratum* (Lyngbye) A.W. Griffiths ex Harvey
Gigartina pistillata (S.G. Gmelin) Stackhouse	*Gelidium repens* Kützing
Gelidiopsis intricata (C. Agardh) Vickers	*Gelidium pinnatifidum* (Hudson) Lyngbye
Grateloupia americana S. Kawaguchi & H.W. Wang	*Gelidium oppositifolium* (C. Agardh) Greville
Wurdemannia miniata (Sprengel) Feldmann & G. Hamel	*Gelidium neglectum* Bory de Saint-Vincent
Carpopeltis maillardii (Montagne & Millardet) Chiang	*Gelidium muricatum* Endlichter
Endocladia muricata (Endlichter) J. Agardh	*Gelidium multicorne* Kützing
Grateloupia filicina (J.V. Lamouroux) C. Agardh	*Gelidium miniatum* Kützing
Callophycus densus (Sonder) Kraft	*Gelidium lanceolatum* Harvey
Osmundea pinnatifida (Hudson) Stackhouse	*Gelidium intricatum* (C. Agardh) Kützing
Gelidiopsis repens (Kützing) Weber-van Bosse	*Gelidium crinitum* (S.G. Gmelin) Kützing
*Pantoneura fabr*iciana (Lyngbye) M.J. Wynne	*Gelidium fastigiatum* Kützing
Gelidiopsis scoparia (Montagne & Millardet) De Toni	*Gelidium decipiens* Montagne
Gelidiopsis pannosa (Grunow) F. Schmitz	*Gelidium gigartinum* (Linnaeus) Lyngbye
Prionitis sternbergii (C. Agardh) J. Agardh	*Gelidium sternbergii (C.* Agardh) Greville
Caulacanthus ustulatus (Mertens ex Turner)	*Gelidium ustulatum* (Mertens ex Turner) J. Agardh
Gelidiopsis variabilis (J. Agardh) Schmitz	*Gelidium variabile* Greville ex J. Agardh
Gracilaria aculeata (K.Hering) Papenfuss	Gelidium constrictum (Turner) Kützing
Polyopes constrictus (Turner) J. Agardh	*Gelidium acrocarpum* Harvey ex Kützing
Plocamium cartilagineum (Linnaeus) P.S. Dixon	*Gelidium cartilagineum* (Linnaeus) Gaillon
Nizymenia australis Sonder	*Gelidium compositum* Kützing

Appendix 6: *Gelidium* species synonymised with species of *Gelidiella.*

Species name	**Synonym**
*Gelidiella ac*erosa (Forsskål) Feldmann & G. Hamel	*Gelidium pulchellum var. setaceum* (Turner) Batters
Gelidiella bornetii (Weber-van Bosse) Feldmann & G. Hamel	*Gelidium bornetii* Weber-van Bosse
Gelidiella calcicola Maggs & Guiry	*Gelidium calcicola* (Maggs & Guiry) R.E. Norris
Gelidiella lubrica (Kützing) Feldmann & G. Hamel	*Gelidium crinale* var. *lubricum* (Kützing) Hauck
Gelidiella lubrica (Kützing) Feldmann & G. Hamel	*Gelidium spiniforme* (J.V. Lamouroux) J.V. Lamouroux
Gelidiella lubrica (Kützing) Feldmann & G. Hamel	*Gelidium lubricum* (Kützing) Trevisan
Gelidiella lubrica (Kützing) Feldmann & G. Hamel	*Gelidium lubricum* (Kützing) Zanardini
Gelidiella ramellosa (Kützing) Feldmann & G. Hamel	*Gelidium corneum* var. *ramellolsum* (Kützing) Harvey
Gelidiella ramellosa (Kützing) Feldmann & G. Hamel	*Gelidium ramelliferum* Kützing
Gelidiella ramellosa (Kützing) Feldmann & G. Hamel	*Gelidium ramellosum* (Kützing) Trevisan
Gelidiella acerosa (Forsskål) Feldmann & G. Hamel	*Gelidium rigidum* (C. Agardh) Greville

Appendix 7: *Gelidium* species synonymised with species of *Ptilophora*

Species name	**Synonym**
Ptilophora pectinata (A. Gepp & E.S. Gepp) R.E. Norris	*Gelidium helenae* Dickinson
Ptilophora rumpii (Dickinson) R.Norris	*Gelidium subcostatum* Okamura
Ptilophora subcostata (Okamura) R.E. Norris	*Gelidium rumpii* Dickinson
Ptilophora prolifera (Harvey) J. Agardh	*Gelidium proliferum* Harvey

Appendix 8: *Ptilophora* species.

P. biserrata (Børgesen) R.E.Norris	*P. leliaertii* E.M.Tronchin & O.De Clerck	*P. subcostata* (Okamura) R.E.Norris
P. coppejansii E.M.Tronchin & O.De Clerck	*P. pectinata* (A.Gepp & E.S.Gepp) R.E.Norris	*P. rumpii* (Dickinson) R.E.Nor
P. diversifolia (Suhr) Papenfuss	*P. mediterranea* (H.Huvé) R.E.Norris	*P. scalaramosa* (Kraft) R.E.Norris
*P. helenae (*Dickinson) R.E.Norris	*P. prolifera* (Harvey) J.Agardh	*P. spissa* (Suhr) Kützing
P. hildebrandtii (Hauck) R.E.Norris	*P. pinnatifida* J.Agardh	*P. rhodoptera* R.E.Norris
P. irregularis (Akatsuka & Masaki) R.E.Norris	*P. pterocladioides* Andriamampandry	

Appendix 9: *Pterocladia* species

Pterocladia mcnabbiana Dawson	*P. lucida* (R. Brown ex Turner) J. Agardh – type	*P. rectangularis* (Lucas) Womersley & Guiry

P. media E.Y. Dawson	*P. heteroplatos* (Børgesen) U. Rao & Kaliaperumal	

Appendix 10: *Pterocladia* species synonymised with species of *Pterocladiella*

Species name	Synonym
Pterocladiella bartlettii (W.R. Taylor) Santelices	*Pterocladia bartlettii* W.R. Taylor
P. bartlettii var. *musciformis* (W.R. Taylor) M.J. Wynne	*Pterocladia bartlettii* var. *musciformis*
P. bulbosa (N.H. Loomis) Santelices	*Pterocladia bulbosa* N.H. Loomis
P. caerulescens (Kützing) Santelices & Hommersand	*Pterocladia caerulescens* (Kützing) Santelices
*P. caespitos*a (Kylin) Santelices	*Pterocladia caespitosa* (Kylin) R.E. Norris
P. caloglossoides (M.A. Howe) Santelices	*Pterocladia caloglossoides* (M.A. Howe) E.Y. Dawson
P. capillacea (S.G. Gmelin) Santelices & Hommersand	*Pterocladia capillacea* (S.G. Gmelin) Bornet
P. capillacea (S.G. Gmelin) Santelices & Hommersand	*Pterocladia densa* Okamura
P. tenuis (Okamura) Shimada, Horiguchi & Masuda	*Pterocladia complanata* N.H. Loomis
P. capillacea (S.G. Gmelin) Santelices & Hommersand	*Pterocladia lindaueri* K. C. Fan
P. melanoidea (Schousboe ex Bornet) Santelices & Hommersand	*Pterocladia melanoidea* (Schousboe ex Bornet) Dawson
P. capillacea (S.G. Gmelin) Santelices & Hommersand	*Pterocladia mexicana* W.R. Taylor
P. nana (Okamura) Shimada, Horiguchi & Masuda	*Pterocladia nana* Okamura
P. capillacea (S.G. Gmelin) Santelices & Hommersand	*Pterocladia okamurae*
P. caloglossoides (M.A. Howe) Santelices	*Pterocladia tenuis* Okamura
P. capillacea (S.G. Gmelin) Santelices & Hommersand	*Pterocladia pyramidale* (Gardner) Dawson
P. capillacea (S.G. Gmelin) Santelices & Hommersand	*Pterocladia pinnata* (Hudson) Papenfuss
P. caerulescens (Kützing) Santelices & Hommersand	*Pterocladia rigida* N.H. Loomis
P. capillacea (S.G. Gmelin) Santelices & Hommersand	*Pterocladia robusta* W.R. Taylor
P. caerulescens (Kützing) Santelices & Hommersand	*Pterocladia tropica* E.Y. Dawson
P. capillacea (S.G. Gmelin) Santelices & Hommersand	*Pterocladia parva* E.Y. Dawson
P. bartlettii (W.R. Taylor) Santelices	*Pterocladia bartlettii* W.R. Taylor
P. bartlettii var. *musciformis* (W.R. Taylor) M.J. Wynne	*Pterocladia bartlettii* var. *musciformis*
P. bulbosa (N.H. Loomis) Santelices	*Pterocladia bulbosa* N.H. Loomis
P. caerulescens (Kützing) Santelices & Hommersand	*Pterocladia caerulescens* (Kützing) Santelices

Appendix 11: *Pterocladia* species have been transefered to *Ptilophora*

Species name	Synonym
Ptilophora pectinata (A. Gepp & E.S. Gepp) R.E. Norris	*Pterocladia pectinata* (A.Gepp & E.S.Gepp) Lucas
Ptilophora pectinata (A. Gepp & E.S. Gepp) R.E. Norris	*Pterocladia lucida f. pectinata* A. Gepp & E.S. Gepp

Appendix 12: *Pterocladiella* species

Pterocladiella australafricanensis Tronchin & Freshwater	*Pterocladiella melanoidea* var. *filamentosa* (Schousboe ex Bornet) M.J. Wynne
Pterocladiella bartlettii (W.R. Taylor) Santelices	*Pterocladiella melanoidea* var. *gracilis* (Feldmann & G.Hamel) M.J. Wynne
Pterocladiella bartlettii var. *musciformis* (W.R. Taylor) Wynne	*Pterocladiella minima* (Guiry & Womersley) Santelices & Hommersand
Pterocladiella beachiae Freshwater in Thomas & Freshwater	*Pterocladiella nana* (Okamura) Shimada, Horiguchi & Masuda C
Pterocladiella bulbosa (N.H. Loomis) Santelices	*Pterocladiella psammophila* Tronchin & Freshwater
Pterocladiella caerulescens (Kützing) Santelices & Hommersand	*Pterocladiella sanctarum* (Feldmann & Hamel) Santelices
Pterocladiella caespitosa (Kylin) Santelices	*Pterocladiella taylorii* (Joly) Santelices
Pterocladiella caloglossoides (M.A. Howe) Santelices	*Pterocladiella tenuis* (Okamura) Shimada, Horiguchi & Masuda
Pterocladiella capillacea (S.G.Gmelin) Santelices & Hommersand	*Pterocladiella yinggehaiensis* Xia & Tseng
Pterocladiella melanoidea (Schousboe ex Bornet) Santelices & Hommersand	

Appendix 13: *Gelidiella* species.

Gelidiella acerosa (Forsskål) Feldmann & G. Hamel	*Gelidiella lubrica* (Kützing) Feldmann & G. Hamel
Gelidiella bornetii (Weber-van Bosse) Feldmann & G. Hamel	*Gelidiella machrisiana* E.Y. Dawson
Gelidiella calcicola Maggs & Guiry	*Gelidiella mexicana* E.Y. Dawson
Gelidiella diuens Sreenivasa Rao & Trivedi	*Gelidiella myrioclada* (Børgesen) Feldmann & G. Hamel
Gelidiella fanii S.-M. Lin C	*Gelidiella nigrescens* (Feldmann) Feldmann & G. Hamel
Gelidiella feldmannii Baardseth	*Gelidiella ramellosa* (Kützing) Feldmann & G. Hamel
Gelidiella hancockii E.Y. Dawson	*Gelidiella rigidiuscula* (Feldmann) Feldmann & G. Hamel
Gelidiella indica Sreenivasa Rao	*Gelidiella tinerfensis* Seoane-Camba
Gelidiella ligulata E.Y. Dawson	

Appendix 14: *Gelidiella* species synonymised with species of *Pterocladia*

Species name	**Synonym**
Pterocladiella minima (Guiry & Womersley) Santelices & Hommersand	*Gelidiella minima* Guiry & Womersley
Pterocladiella taylorii (Joly) Santelices	*Gelidiella sanctarum* Feldmann & G. Hamel
Pterocladiella sanctarum (Feldmann & Hamel) Santelices	*Gelidiella taylori* A.B. Joly

Appendix 15: *Parviphycus* species and their synonyms

Species name	**Synonym**
Parviphycus adnatus (E.Y. Dawson) B. Santelices	*Gelidiella adnata* E.Y. Dawson
Parviphycus antipai (Celan) B. Santelices	*Gelidiella antipai* Celan
Parviphycus antipai (Celan) B. Santelices	*Gelidiella stichidiospora* E.Y. Dawson
Parviphycus felicinii C.Perrone & C.I.Delle Foglie	
Parviphycus pannosus (Feldmann) G.Furnari	*Gelidiella pannosa* Feldmann & G. Hamel
Parviphycus setaceus (Feldmann) Afonso-Carrillo, Sanson,.Sangil & Diaz-Villa	*Gelidiella setacea* (Feldmann) Feldmann & G. Hamel

Parviphycus trinitatensis (W.R.Taylor) M.J.Wynne	*Gelidiella trinitatensis* W.R. Taylor
Parviphycus womersleyanus (Kraft & I.A. Abbott) B. Santelices	*Gelidiella womersleyana* Kraft & I.A. Abbott

Appendix 16: List of the Gelidiales collected from coastline of Malaysia.

Code number	Species name	Collection date	Locality
PSM12500	*Gelidiella acerosa*	30.Dec.2009	Port Dickson(2˚24 ' 54" N / 101˚51 ' 10 " E)
PSM12514	*Gelidiella acerosa*	28. Feb. 2010	Port Dickson(2˚24 ' 54" N / 101˚51 ' 10 " E)
PSM12584	*Gelidiella acerosa*	2.Aug.2011	Teluk Kemang, Port Dickson(2º 24 ' 54" N; 101º 51'20" E)
PSM12588	*Gelidiella acerosa*	2.Aug.2011	Teluk Kemang, Port Dickson(2º 24 ' 54" N; 101º 51'20" E)
PSM12640	*Gelidiella acerosa*	17. Feb. 2012	Pantai Bukit Kelang- Terrenganu (5 ° 48' 5.84" N; 102 ° 36' 17" E).
PSM12643	*Gelidiella acerosa*	17.Feb.2012	Panti Chendering, Terrenganu(5 ° 16' 9.63" N; 103 ° 11' 18.62" E)
PSM12552	*Gelidium sp.2*	9. 11. 2010	Kampung Dandulite (5˚59 '43.45" N / 117˚54 ' 50.70 " E) Sandakan,
PSM12589	*Gelidium sp.2*	2.Aug.2011	Teluk Kemang, Port Dickson(2º 24 ' 54" N; 101º 51'20" E)
PSM12642	*Gelidium sp.2*	17. Feb. 2012	Pantai Bukit Kelang- Terrenganu(5 ° 48' 5.84" N; 102 ° 36' 17" E)
PSM12646	*Gelidium sp.2*	17. Feb. 2012	Panti Chendering, Terrenganu(5 ° 16' 9.63" N; 103 ° 11' 18.62" E)
PSM12493	*Gelidium sp.1*	8.Sept.2009	Pulau Pinang - Batu Feringi(5 ° 28' 51" N; 100 ° 15' 15" E)
PSM12494	*Gelidium sp.1*	8.Sept.2009	Pulau Pinang - Batu Feringi(5 ° 28' 51" N; 100 ° 15' 15" E)
PSM12508	*Gelidium sp.1*	13.Feb.2010	Pulau Pinang - Batu Feringi(5 ° 28' 51" N; 100 ° 15' 15" E)
PSM12524	*Gelidium sp.1*	27.Apr.2010	Pulau Pinang - Batu Feringi(5 ° 28' 51" N; 100 ° 15' 15" E)
PSM12625	*Gelidium sp.1*	5.Feb.2012	Pulau Pinang , Monky tree garden

PSM12630	*Gelidium sp.1*	6.Feb.2012	Pulau Pinang Kim Bina Negara (5˚, 18,' 0.52" N /100˚, 11' ,4" E)

Appendix 16: List of the Gelidiales collected from coastline of Malaysia (Continue)

Code number	Species name	Collection date	Locality
PSM12631	*Gelidium sp.1*	6.Feb.2012	Pulau Pinang Kim Bina Negara (5˚, 18,' 0.52" N /100˚, 11' ,4" E)
PSM12632	*Gelidium sp.1*	6.Feb.2012	Pulau Pinang Kim Bina Negara (5˚, 18,' 0.52" N /100˚, 11' ,4" E)
PSM12637	*Gelidium sp.1*	16.Feb.2012	Pantai Komasi, Terrenganu
PSM12641	*Gelidium sp.1*	17. Feb. 2012	Pantai Bukit Kelang- Terrenganu(5 ° 48' 5.84" N; 102 ° 36' 17" E)
PSM12653	*Gelidium sp.1*	17.Feb.2012	Panti Chendering, Terrenganu(5 ° 16' 9.63" N; 103 ° 11' 18.62" E)
PSM12499	*Gelidium cf. crinale var. perpusillum*	20. Oct.2009	Port Dickson(2˚24 ' 54" N / 101˚51 ' 10 " E)
PSM12503	*Gelidium cf. crinale var. perpusillum*	30.Dec.2009	Port Dickson(2˚24 ' 54" N / 101˚51 ' 10 " E)
PSM12517	*Gelidium cf. crinale var. perpusillum*	28.Feb.2010	Port Dickson(2˚24 ' 54" N / 101˚51 ' 10 " E)
PSM12522	*Gelidium cf.crinale var. perusillum*	11.Apr.2010	Pulau Besar (2˚,06,' 57" N 102˚, 19' ,54" E)
PSM12577	*Gelidium cf. crinale var. perpusillum*	2.Aug.2011	Teluk Kemang, Port Dickson(2º 24 ' 54" N; 101º 51'20" E)
PSM12578	*Gelidium cf.crinale var. perpusillum*	2.Aug.2011	Teluk Kemang, Port Dickson(2º 24 ' 54" N; 101º 51'20" E)
PSM12590	*Gelidium cf.crinale var. perpusillum*	29.Aug.2011	Teluk Kemang, Port Dickson(2º 24 ' 54" N; 101º 51'20" E)
PSM12591	*Gelidium cf.crinale var. perpusillum*	29.Aug.2011	Teluk Kemang, Port Dickson(2º 24 ' 54" N; 101º 51'20" E)
PSM12609	*Gelidium cf.crinale var. perpusillum*	25.Nov.2011	Teluk Kemang, Port Dickson(2º 24 ' 54" N; 101º 51'20" E)
PSM12565	*Gelidium cf. crinale var. perpusillum)*	27.Apr.2011	Port Dickson(2˚24 ' 54" N / 101˚51 ' 10 " E)

Appendix 16: List of the Gelidiales collected from coastline of Malaysia (Continue)

Code number	Species name	Collection date	Locality
PSM12554	*Gelidium sp.*	9. Nov. 2010	Kampung Dandulite (5˚59 '43.45" N / 117˚54 ' 50.70 " E) Sandakan,
PSM12560	*Gelidium sp.*	13.Feb.2011	Panti Chendering, Terrenganu(5 ° 16' 9.63" N; 103 ° 11' 18.62" E)
PSM12652	*Gelidium sp.*	17.Feb.2012	Panti Chendering, Terrenganu(5 ° 16' 9.63" N; 103 ° 11' 18.62" E)
PSM12657	*Gelidium sp.*	25.Mar.2012	Kampong Budaya(1 ° 43' 13.68" N; 110 ° 118' 48.27" E)Kuching, Sarawak
PSM12592	*Gelidium sp.* - silde	29.Aug.2011	Teluk Kemang, Port Dickson(2º 24 ' 54" N; 101º 51’20" E)
PSM12593	*Gelidium sp.*- silde	29.Aug.2011	Teluk Kemang, Port Dickson(2º 24 ' 54" N; 101º 51’20" E)
PSM12553	*Gelidium sp.1*	9. Nov 2010	Kampung Dandulite (5˚59 '43.45" N / 117˚54 ' 50.70 " E) Sandakan
PSM12667	*P. caerulescens*	8.Jun.2012	Port Dickson(2˚24 ' 54" N / 101˚51 ' 10 " E)
PSM12668	*P. caerulescens*	8.Jun012	Port Dickson(2˚24 ' 54" N / 101˚51 ' 10 " E)
PSM12664	*P. bartletti*	8.Jun.2012	Port Dickson(2˚24 ' 54" N / 101˚51 ' 10 " E)
PSM12665	*P. bartletti*	8.Jun.2012	Port Dickson(2˚24 ' 54" N / 101˚51 ' 10 " E)
PSM12662	*P. caerulescensed*	8.Apr.2012	Port Dickson(2˚24 ' 54" N / 101˚51 ' 10 " E)
PSM12518	*Parviphycus sp.*	28.Feb.2010	Port Dickson(2˚24 ' 54" N / 101˚51 ' 10 " E)
PSM12534	*Parviphycus sp.*	10.Jun.2010	Port Dickson(2˚24 ' 54" N / 101˚51 ' 10 " E)
PSM12666	*P. beachiae*	8.Jun.2012	Port Dickson(2˚24 ' 54" N / 101˚51 ' 10 " E)

Appendix 16: List of the Gelidiales collected from coastline of Malaysia (Continue)

Code number	Species name	Collection date	Locality
PSM12661	*P. beachiae* - spore cultured	18.Apr.2012	Port Dickson(2˚24 ' 54" N / 101˚51 ' 10 " E)
PSM12542	*Parviphycus sp.*	12. July 2010	Teluk Kemang, Port Dickson(2º 24 ' 54" N; 101º 51'20" E)
PSM12579	*Parviphycus sp.*	2.Aug.2011	Teluk Kemang, Port Dickson(2º 24 ' 54" N; 101º 51'20" E)
PSM12581	*Parviphycus sp.*	2.Aug.2011	Teluk Kemang, Port Dickson(2º 24 ' 54" N; 101º 51'20" E)
PSM12610	*Parviphycus sp.*	25.Nov.2011	Teluk Kemang, Port Dickson(2º 24 ' 54" N; 101º 51'20" E)
PSM12611	*Parviphycus sp.*	25.Nov.2011	Teluk Kemang, Port Dickson(2º 24 ' 54" N; 101º 51'20" E)
PSM12639	*parviphycus sp.*	17.Feb 2012	Pantai Bukit Kelang- Terrenganu (5 ° 48' 5.84" N; 102 ° 36' 17" E).
PSM12658	*Parviphycus sp.*	25.Marr	Kampong Budaya(1 ° 43' 13.68" N; 110 ° 118' 48.27" E)Kuching, Sarawak
PSM12660	*Parviphycus sp.*	25.Mar201	Kampong Budaya(1 ° 43' 13.68" N; 110 ° 118' 48.27" E)Kuching, Sarawak
PSM12663	*Parviphycus sp.*	8.Jun.2012	Port Dickson(2˚24 ' 54" N / 101˚51 ' 10 " E)
PSM12672	*Parviphycus sp.*	3. Aug.2012	Palua Manukan (5˚32' 27.68 " N, 116 ˚00 '11.34 " E) Kota Kinabalu, Sabah
PSM12673	*Parviphycus sp.*	3. Aug.2012	Palua Manukan (5˚32' 27.68 " N, 116 ˚00 '11.34 " E) Kota Kinabalu, Sabah
PSM12674	*Parviphycus sp.*	3. Aug.2012	Palua Manukan (5˚32' 27.68 " N, 116 ˚00 '11.34 " E) Kota Kinabalu, Sabah
PSM12675	*Parviphycus sp.*	3. Aug.2012	Palua Manukan (5˚32' 27.68 " N, 116 ˚00 '11.34 " E) Kota Kinabalu, Sabah
PSM12676	*Parviphycus sp.*	3. Aug.2012	Palua Manukan (5˚32' 27.68 " N, 116 ˚00 '11.34 " E) Kota Kinabalu, Sabah
PSM12677	*Parviphycus sp.*	3. Aug.2012	Palua Manukan (5˚32' 27.68 " N, 116 ˚00 '11.34 " E) Kota Kinabalu, Sabah

Appendix 16: List of the Gelidiales collected from coastline of Malaysia (Continue)

Code number	Species name	Collection date	Locality
PSM12563	*Parviphycus sp.*	27.Apr.2011	Teluk kemang-Port Dickson(2º 24 ' 54" N; 101º 51'20" E)
PSM12647	*Parviphycus sp.*	17.Feb.2012	Panti Chendering, Terrenganu(5 ° 16' 9.63" N; 103 ° 11' 18.62" E)
PSM12523	*parviphycus sp.*- silde	11.Apr.2010	Pulau Besar(2˚,06,' 57" N 102˚, 19' ,54" E)
PSM12594	*Parviphycus sp.*- silde	29.Aug.2011	Teluk Kemang, Port Dickson(2º 24 ' 54" N; 101º 51'20" E)
PSM12597	*parviphycus sp.*- silde	29.Aug.2011	Teluk Kemang, Port Dickson(2º 24 ' 54" N; 101º 51'20" E)
PSM12612	*Parvyphycus sp.*	25.Nov.2011	Teluk Kemang, Port Dickson(2º 24 ' 54" N; 101º 51'20" E)
PSM12519	*Pterocladiella beachiae*	11.Apr.2010	Pulau Besar (2˚,06,' 57" N 102˚, 19' ,54" E)
PSM12520	*Pterocladiella beachiae*	11.Apr.2010	Pulau Besar (2˚,06,' 57" N 102˚, 19' ,54" E)
PSM12604	*Pterocladiella* sp. nov. 2	29.Aug.2011	Teluk Kemang, Port Dickson(2º 24 ' 54" N; 101º 51'20" E)
PSM12563	*Parviphycus sp.*	27.Apr.2011	Teluk kemang-Port Dickson(2º 24 ' 54" N; 101º 51'20" E)
PSM12523	*parviphycus sp.*- silde	11.Apr.2010	Pulau Besar(2˚,06,' 57" N 102˚, 19' ,54" E)
PSM12594	*Parviphycus sp.*- silde	29.Aug.2011	Teluk Kemang, Port Dickson(2º 24 ' 54" N; 101º 51'20" E)
PSM12597	*parviphycus sp.*- silde	29.Aug.2011	Teluk Kemang, Port Dickson(2º 24 ' 54" N; 101º 51'20" E)
PSM12612	*Parvyphycus sp.*	25.Nov.2011	Teluk Kemang, Port Dickson(2º 24 ' 54" N; 101º 51'20" E)
PSM12519	*Pterocladiella beachiae*	11.Apr.2010	Pulau Besar (2˚,06,' 57" N 102˚, 19' ,54" E)

Appendix 16: List of the Gelidiales collected from coastline of Malaysia (Continue)

Code number	Species name	Collection date	Locality
PSM10810	*Pterocladiella beachiae*	8.Oct.2009	Pulau Pinang - Batu Feringi(5 ° 28' 51" N; 100 ° 15' 15" E
PSM12520	*Pterocladiella beachiae*	11.Apr.2010	Pulau Besar (2˚,06,' 57" N 102˚, 19' ,54" E)
PSM12604	*Pterocladiella* sp. nov. 2	29.Aug.2011	Teluk Kemang, Port Dickson(2º 24 ' 54" N; 101º 51'20" E)
PSM10811	*Pterocladiella beachiae*	8.Oct.2009	Pulau Pinang - Batu Feringi(5 ° 28' 51" N; 100 ° 15' 15" E)
PSM12497	*Pterocladiella beachiae*	08.Sept.2009	Pulau pinang (5 ° 28' 51" N; 100 ° 15' 15"E)
PSM12540	*Pterocladiella beachiae*	12. July 2010	Teluk Kemang, Port Dickson(2º 24 ' 54" N; 101º 51'20" E)
PSM12546	*Pterocladiella beachiae*	8.Nov.2010	Pulau Nunuyang Laut, Sandakan, Sabah (5 ° 55' 24" N; 118 ° 05' 28" E)
PSM12618	*Pterocladiella beachiae-*	25.Nov.2011	Teluk Kemang, Port Dickson(2º 24 ' 54" N; 101º 51'20" E)
PSM12538	*Pterocladiella beachiae*	12. July 2010	Teluk Kemang, Port Dickson(2º 24 ' 54" N; 101º 51'20" E)
PSM12582	*Pterocladiella beachiae*	2.Aug.2011	Teluk Kemang, Port Dickson(2º 24 ' 54" N; 101º 51'20" E)
PSM12595	*Pterocladiella beachiae*	29.Aug.2011	Teluk Kemang, Port Dickson(2º 24 ' 54" N; 101º 51'20" E)
PSM12515	*Pterocladiella caerulescens*	28.Feb.2010	Port Dickson(2˚24 ' 54" N / 101˚51 ' 10 " E)
PSM12537	*Pterocladiella caerulescens*	12. July 2010	Teluk Kemang, Port Dickson(2º 24 ' 54" N; 101º 51'20" E)
PSM12576	*Pterocladiella caerulescens*	2.Aug.2011	Teluk Kemang, Port Dickson(2º 24 ' 54" N; 101º 51'20" E)
PSM12596	*Pterocladiella caerulescens*	29.Aug.2011	Teluk Kemang, Port Dickson(2º 24 ' 54" N; 101º 51'20" E)
PSM12527	*Pterocladiella caerulescens*	10.Jun.2010	Port Dickson(2˚24 ' 54" N / 101˚51 ' 10 " E)
PSM12501	*Pterocladiella caerulescens*	30.Dec.2009	Port Dickson(2˚24 ' 54" N / 101˚51 ' 10 " E)
PSM12603	*Pterocladiella sp . nov.2*	29.Aug.2011	Teluk Kemang, Port Dickson(2º 24 ' 54" N; 101º 51'20" E)

Appendix 17: GenBank accession numbers of Pterocladiaceae species, Malaysian sequences of Pterocladiaceae and outgroups used for phylogenetic analyses.

			GenBank accession number		
Species	**Locality**	**Voucher**	***rbc*L**	***cox*I**	**LSU**
Aphanta pachyrhriza	Sodwan Bay S. Africa	SA04-50	EF190244	-	EF190257
Aphanta pachyrhriza	Sodwan Bay S. Africa	Gsp- Sodw	EF190245	-	-
Aphanta sp.	Port Dickson Malaysia	PSM12539	-	166-coxI	-
Aphanta sp.	Port Dickson Malaysia	PSM12586	257-rbcL	257-coxI	257-LSU
Aphanta sp.	Port Dickson Malaysia	PSM12586	257-2-rbcL	-	-
Aphanta sp.	K. K., Sabah Malaysia	PSM12587	-	262-coxI	262-LSU
Aphanta sp.	K. K., Sabah Malaysia	PSM12587	-	262-2-coxI	-
Aphanta sp.	Port Dickson Malaysia	PSM12679	-	357-coxI	357-LSU
Gelidiella acerosa	New Caledonia	-	EU146836	-	-
G. acerosa	Korea	-	-	HM102421	-
G. acerosa	Tanzania	14.vii.2001	-	-	FJ215875
Gelidium australe	Canada	GWS001558	-	-	DQ343682
Gelidium japonicum	Japan	G2190	HM629830	-	-
Gelidium japonicum	Taiwan	-	-	-	AF521185
G. divaricatum	Korea	G4025	-	HM629865	-
Pterocladia lucida	New Zealand	ASD141	AY648025	-	-
Capreolia implexa	Victoria, Australia		-	-	AF039545
P. lucida	New Zealand	LHI-12	AY352423	-	AF039550
P. lucida	New Zealand	-	U01048	-	AF419118
Pterocladiella bartlettii	Pulau Pinang, Malaysia	PSM12495A	KC209059	KC209081	PSM12495A
P. bartlettii	Pulau Pinang, Malaysia	PSM12495B	KC209061	-	-
P. bartlettii	Pulau Pinang, Malaysia	PSM12496A	KC209060	KC209083	-
P. bartlettii	Pulau Pinang, Malaysia	PSM12496B	KC209058	-	-
P. bartlettii	Port Dickson, Malaysia	PSM12564A	KC209062	KC209084	PSM12564A
P. bartlettii	Teluk Kemang, Malaysia	PSM12664	-	KC209082	-
P. bartlettii	Port Dickson, Malaysia	PSM12564B	KC209063	-	-
P. bartlettii	Cahuita, Costa Rica	-	AB305806	-	
P. bartlettii	Cahuita, Costa Rica	CR8	-	-	AF296516
P. bartlettii	Texas, USA	-	AF305807	-	AF296515
P. beachiae	Cahuita, Costa Rica	CR12	AF305811	-	AF296514
P. beachiae	Bacas, Panama	Pan07-001	JN114113	-	-
P. beachiae	Cahuita, Costa Rica	Pcaer-CR12	-	HQ412477	-
P. beachiae	Bacas, Panama	PHYKOS-3217	-	HQ412480	-
P. beachiae	Bacas, Panama	PHYKOS-3226	HQ412500	HQ412482	-
P. beachiae	Bacas, Panama	PHYKUS-3213	-	HQ412478	-
P. beachiae	Bacas , Panama	PHYKUS-3216	-	HQ412479	-
P. beachiae	Bacas, Panama	PHYKUS-3229	HQ412499	HQ412481	-
P. beachiae	Pulau Pinang, Malaysia	PSM12497	KC209075	KC209093	31-LSU
P. beachiae	Pulau Besar, Malaysia	PSM12519	KC209073	KC209094	127C_LSU
P. beachiae	Pulau Besar, Malaysia	PSM12520	KC209074	-	
P. beachiae	Teluk Kemang, Malaysia	PSM12618A	KC209076	KC209095	
P. beachiae	Teluk Kemang, Malaysia	PSM12618B	KC209077	-	
P. caerulescens	Hawaiian Island, USA	Pcaer-HI	-	HQ412475	
P. caerulescens	Hawaiian Island, USA	-	-	-	AF296513
P. caerulescens	Hawaiian Island, USA	Pcaer-SB	-	HQ412476	-
P. caerulescens	Port Dickson, Malaysia	PSM12501	KC209069	KC209097	-
P. caerulescens	Port Dickson, Malaysia	PSM12530	KC209071	-	-
P. caerulescens	Port Dickson, Malaysia	PSM12531	KC209070	KC209098	-
P. caerulescens	Port Dickson, Malaysia	PSM12662	KC209072	KC209096	339-LSU
P. caerulescens	Hawaiian Island, USA	PtcaerSB	EF190250	-	-

Appendix 17: GenBank accession numbers of Pterocladiaceae species, Malaysian sequences of Pterocladiaceae and outgroups used for phylogenetic analyses (Continue).

			GenBank accession number		
Species	**Locality**	**Voucher**	***rbc*L**	***cox*I**	**LSU**
P. australafricanensis	Kwazulu-Natal, South Africa	SA04-045	EF190246	HQ412472	EF190259
P. australafricaennsis	Mozambique	SA04-095	EF190248	-	-
P. caerulescens	Mozambique	SA04-072	-	HQ412473	
P. caerulescens	Hawaiian Island, USA	-	AF305805	-	-
P. caloglossoides	NSW, Australia	NSW-1	AY352422	-	AY359962
P. capillacea	NSW, Australia	NSW-3	AY352421	-	-
P. capillacea	South Korea	P1251	-	HM629885	-
P. capillacea	Japan	-	AB023850	-	-
P. capillacea	Italy		-	-	AF308797
P. capillacea	USA	-	-	-	AF039549
Pterocladiella sp. nov.2	Teluk Kemang, Malaysia	PSM12599	KC209065	KC209087	-
Pterocladiella sp. nov.2	Teluk Kemang, Malaysia	PSM12600	KC209064	KC209088	-
Pterocladiella sp. nov.2	Teluk Kemang, Malaysia	PSM12601A	KC209067	KC209089	-
Pterocladiella sp. nov.2	Teluk Kemang, Malaysia	PSM12601B	KC209068	KC209085	-
Pterocladiella sp. nov.2	Pulau Pinang, Malaysia	PSM12628	KC209066	KC209086	300-LSU
P. melanoidea	Spain	Mallorca	U01046	-	AF039548
Ptilophora coppejansii	Kwazulu-Natal. S. Africa	DWF-2002	-	-	AF521118
P. nana	Korea	P1176	GU731223	-	-
P. nana	Korea	P1352	GU731224	-	-
Pterocladiella sp. nov.1	Port Dickson, Malaysia	PSM12504	KC209078	KC209090	-
Pterocladiella sp. nov. 1	Port Dickson, Malaysia	PSM12505A	KC209080	KC209091	-
Pterocladiella sp. nov. 1	Port Dickson, Malaysia	PSM12598	-	KC209092	-
Pterocladiella sp. nov.1	Port Dickson, Malaysia	PSM12505B	KC209079	-	-
P. psammophila	K.-Natal, South Africa	-	-	HQ412486	-
P. psammophila	K-Natal, South Africa	Pcaer2	-	HQ412484	-
P. psammophila	K-Natal, South Africa	Pcaer3	EF190256	HQ412485	-
P. psammophila	K-Natal, South Africa	SA04-042	EF190255	HQ412483	EF190260
P. tenuis	Korea	P0868	GU731220	-	-
P. tenuis	Korea	P0929	GU731221	-	-

Appendix 18: GenBank accession numbers of Gelidiaceae species, Malaysian sequences of Gelidiaceae and outgroups used for phylogenetic analyses.

			GenBank accession number		
Species	**location**	**Voucher code**	***rbcL***	***cox*I**	**LSU**
Acanthopeltis longiramulosa	South Korea	A0332	HM629843	-	-
Gelidium coreanum	Korea	CNU000127	JQ340391	-	-
Gelidium asperum	Australia	Ptlons2	AY350872		
Gelidium prostratum	Korea	CNU010848	JQ340415	-	-
Gelidium japonicum	Japan	G2190	HM629830	-	-
Gelidium chilense	Chile	-	AF305800	-	-
Gelidium rex	Chile	-	AF305801	-	-
Gelidium omanense	Oman	1609	AY346460	-	-
Gelidium omanense	Oman	1009	AY346461	-	-
Gelidium pluma	USA	-	AF501288	-	-
Gelidium pluma	USA	-	AF522367	-	-
Gelidium longipes	New Zealand	-	AY648021	-	-
Gelidium crinale	South Korea	G2656	HM629823	-	-
Gelidium pusillum	Japan	-	AB017679	-	-
Gelidium microphyllum	New Zealand	-	AY648022	-	-
Gelidium rex	Chile	G3599	HM629835	-	-
Gelidium indonesianum	Korea	-	JF330222	-	-
Gelidium indonesianum	Korea	-	JF330224	-	-
Gelidium amansii	South Korea	G2	DQ787586	-	-
Gelidium americanum	USA	-	L22459	-	-
Gelidium pteridifolium	South Africa	SA04-110	EF190253	-	-
Gelidium profundum	South Africa	SA04-129	EF190251	-	-
Gelidium abbottiorum	South Africa	SA04-109	EF190254	-	-
Gelidium spinosum	Spain	G2449	HM629837	-	-
Gelidium latifolium	USA	-	U00116	-	-
Gelidium canariensis	USA	-	L22460	-	-
Gelidium declerckii	South Africa	S14	AY350775	-	-
Gelidium corneum	Morocco	G2583	HM629821	-	-
Gelidium sesquipedale	USA	-	L22071	-	-
Gelidium isabelae	South Africa	0962	AF305798	-	-
Gelidium microdonticum	Costa Rica	-	AF305799	-	-
Gelidium microphyllum	New Zealand		AY648022		
Gelidium pristoides	South Africa	G3598	HM629834	-	-
Suhria vittata	USA	-	AF501289	-	-
Gelidium pusillum	France	G3579	HM629832	-	-
Gelidium pusillum	United Kingdom	-	JX096526	-	-
Gelidium pusillum	USA	-	GPU00999	-	-
Beckerella helenae	South Africa	-	AY344045	-	-
Ptilophora leliaertii	USA	-	U16834	-	-
Ptilophora coppejansii	South Africa	-	AF522366	-	-
Ptilophora rhodoptera	South Africa	-	AF522365	-	-
Ptilophora pinnatifida	South Africa	-	AF522361	-	-
Ptilophora mediterranea	Greece	Isotype	AF522360	-	-
Ptilophora subcostata	USA	-	U16835	-	-
Beckerella pectinata	Australia	-	AY344043	-	-
Gelidium pristoides	South Africa		HM629834		
Gelidium hommersandii	Australia	NSW-13	AY352420	-	-
Gelidium caulacantheum	New Zealand	-	AY648020	-	-
Gelidium caulacantheum	New Zealand	-	AY648018	-	-
Gelidium hommersandii	New Zealand	-	U01043	-	-
Capreolia implexa	New Zealand	-	AY648013	-	-
Capreolia implexa	USA	-	L22456	-	-
Capreolia implexa	New Zealand	-	AY648012	-	-
Gelidium divaricatum	South Korea	G2098	HM629824	-	-
Gelidium divaricatum	South Korea	G4058	HM629826	-	-
Rhodymenia divaricatum	Puerto Rico	-	EU670597	-	-
Gigartina skottsbergii	Antarctica	-	AF146206	-	-
Gelidium sp.nov.1	Pulau Pinang Malaysia	PSM12493	18 rbcL		-
Gelidium sp.nov.1	Pulau Pinang Malaysia	PSM12494	19 rbcL	19cox1	-
Gelidium sp.nov.1	Pulau Pinang Malaysia	PSM12632	298 rbcL	298cox1	-

Appendix 18: GenBank accession numbers of Gelidiaceae species, Malaysian sequences of Gelidiaceae and outgroups used for phylogenetic analyses (Continue).

			GenBank accession number		
Species	**location**	**Voucher code**	***rbcL***	***cox*I**	**LSU**
Gelidium sp.nov.1	Langkawi Malaysia	PSM12524	140 rbcL		-
Gelidium sp.nov.1	Terrenganu , Malaysia	PSM12641	309 rbcL	309cox1	-
Gelidium sp nov.1	Terrenganu , Malaysia	PSM12641	-	309-2cox1	-
Gelidium sp.nov.1	Pulau Pinang Malaysia	PSM12625	-	301-1cox1	-
Gelidium sp.nov.1	Pulau Pinang Malaysia	PSM12625	-	301-3 cox1	-
Gelidium sp.nov.1	Terrenganu , Malaysia	PSM12637	-	317-cox1	-
Gelidium sp.nov.1	Terrenganu , Malaysia	PSM12653	-	330 cox1	-
G. cf. *crinael.*var. *perpusillum*	Pulau Besar, Malaysia	PSM12522	129 rbcL	-	-
*G.*cf. *crinale* var. *perpusillum*	Port Dickson Malaysia	PSM12590	275 rbcL	275-1 cox1	-
G. cf. *crinale* var. *perpusillum*	Port Dickson Malaysia	PSM12590		275-2 cox1	-
G. cf. *crinael.*var. *perpusillum*	Port Dickson Malaysia	PSM12591	276 rbcL	-	-
*G.*cf *.crinale* var. *perpusillum*	Port Dickson Malaysia	PSM12517	123 rbcL	-	-
G. cf. *crinale* var. *perpusillum*	Port Dickson Malaysia	PSM12503	101 rbcL	-	-
Gelidium sp.nov.2	T. Kemang, Malaysia	PSM12589	264-1 rbcL	264-1 cox1	-
Gelidium sp.nov.2.	T. Kemang, Malaysia	PSM12589	264-2 rbcL	-	-
Gelidium sp.nov.2	T. Kemang, Malaysia	PSM12605	-	273 cox1	-
Gelidium sp.nov.2	Terrenganu, Malaysia	PSM12642	312 rbcL	312cox1	-
Gelidium sp.nov.2	Terrenganu, Malaysia	PSM12646	318rbcL	318 cox1	-
Gelidium pluma	USA	ARS00824	-	HQ422688	-
Gelidium eucorneum	South Korea	G6112	-	HM629854	-
Gelidium prostratum	South Korea	CNU011879	-	JQ340457	-
Gelidium jejuensis	South Korea	CNU011862	JQ340406	JQ340445	-
Gelidium vagum	South Korea	G2229	-	HM629878	-
Gelidium vagum	South Korea	G2918	-	HM629880	-
Gelidium divaricatum	South Korea	G4025	-	HM629865	-
Gelidium divaricatum	South Korea	G4058	-	HM629866	-
Gelidium caulacantheum	New Zealand	G2447	-	HM629859	-
Gelidium caulacantheum	New Zealand	G3582	-	HM629860	-
Gelidium elegans	South Korea	P1113	-	HM629868	-
Gelidium pacificum	Japan	G2197	-	HM629871	-
Gelidium purpurascens	USA	G2627	-	HM629873	-
Gelidium robustum	Mexico	G4750	HM629836	HM629876	-
Gelidium indonesianum	Indonesia	G2726	-	JF330205	-
Gelidium indonesianum	Indonesia	G2729	-	JF330207	-
Gelidium corneum	South Korea	G2483	-	HM629861	-
Gelidium minimum	South Korea	CNU011897	JQ340414	JQ340452	-
Gelidium crinale	South Korea	GNU009062		JX096536	
Gelidium crinale	Namehae, S.Korea	GNU009065		JX096538	
Gelidium crinale	Namehae, S.Korea	GNU009063		JX096537	
Gelidium crinale	Joencheon , S. Korea	GNU096539		JX096539	
Gelidium crinale	Ieiu, south Korea	G3923		JX096540	
Gelidium crinale	Hongkong	Honkong21		JX096532	
Gelidium crinale	Hongkong	G2710		JX096534	
Gelidium crinale	Qingdao, China	GNU00179		JXO96528	
Gelidium crinale	Hanian, China	P1576		JX096530	
Gelidium crinale	Cadiz, Spain	G6359		JX096541	
Gelidium crinale	Sidmoth, UK	G6292		JX096542	
Gelidium crinale	Texas, USA	LB2321		JX096543	
Gelidium pusillum	France	G3579		HM629872	
Gelidium pusillum	Sidmouth, UK	G6301		JX096550	
Gelidium pusillum	Ilfracombe, UK	G6248		JX096546	
Gelidium pusillum	Sidmoth, UK	G6304		JX096551	
Gelidium pusillum	Ilfracombe, UK	G6272		JX096549	
Gelidium pusillum	Cadiz , Spain	G6315		JX096544	
Gelidium pusillum	Cadiz, Spain	G6319		JX096545	
Gelidium pusillum	Ilfracombe, UK	G6254		JX096547	
Gelidium pusillum	Ilfracombe, UK	G6262		JX096548	
Gelidium crinale	South Korea	GNU009062		JX096536	
Gelidium crinale	Namehae, S.Korea	GNU009065		JX096538	
Gelidium crinale	Namehae, S.Korea	GNU009063		JX096537	
Ptilophora rhodoptera	South Africa	-	-	-	AF521183
Ptilophora diversifolia	South Africa	-	-	-	AF521182
Ptilophora coppejansii	South Africa	-	-	-	AF521184

Appendix 18: GenBank accession numbers of Gelidiaceae species, Malaysian sequences of Gelidiaceae and outgroups used for phylogenetic analyses (Continue).

			GenBank accession number		
Species	**location**	**Voucher code**	***rbcL***	***cox*I**	**LCU**
Beckerella helenae	South Africa	-	-	-	AY345880
Ptilophora leliaertii	USA	-	-	-	AF039547
Ptilophora mediterranea	Greece	-	-	-	AF521179
Ptilophora pinnatifida	South Africa	-	-	-	AF521180
Ptilophora prolifera	Australia	-	-	-	AF296511
Ptilophora hildebrandtii	South Africa	-	-	-	AF521178
Ptilophora subcostata	USA	-	-	-	AF039546
Gelidium caulacantheum	New Zealand	-	-	-	AF039544
Capreolia implexa	New Zealand	-	-	-	AF039545
Gelidium serrulatum	Veuzeula				AF039538
Gelidium floridanum	Costa Rica	GR13	-	-	AF296510
Gelidium floridanum	USA	-	-	-	AF039537
Gelidium serrulatum					AF039536
Gelidium americanum	USA	-	-	-	AF039536
Gelidium latifolium	France	-	-	-	AF039540
Onikusa pristoides	USA	-	-	-	AF039541
Suhria vittata	South Africa	-	-	-	AF419119
Gelidium vittatum	South Africa	Suhr	-	-	EF190258
Gelidium pusillum	Norway	-	-	-	AF039542
Gelidium japonicum	Taiwan	-	-	-	AF521185
Gelidium crinale	Spain	1126	-	-	AF308784
Ptilophora rhodoptera	South Africa	-	-	-	AF521183

Appendix 19: GenBank accession numbers of Gelidiellaceae species, Malaysian sequences of Gelidiellaceae and outgroups used for phylogenetic analyses.

			GenBank accession number	
Species	**location**	**Voucher code**	***rbcL***	***coxI***
Gelidiella acerosa	Puerto Rico		AF305810	
Gelidiella acerosa	Costa Rica		AF305812	
Gelidiella acerosa	Tanzania		EU146837	
Gelidiella acerosa	Australia	Palm Cove	AF329822	
Gelidiella acerosa	Australia	LHI-6	AY352424	
Gelidiella acerosa	New Caledonia		EU146836	
Gelidiella acerosa	Hawaii, USA		L22457	
Gelidiella fanii	Thailand	G5177	HM026538	HM026518
Gelidiella fanii	Thailand	G5145	HM026539	
Gelidiella fanii	Indonesia	G5239	HM026541	
Gelidiella ligulata	Japan		AB017678	
Gelidiella ramellosa	Australia	J10.i.20007	FJ215879	
Parviphycus antipai	Australia	LHI-5	AY352425	
Parviphycus pannosus	France	GPannTYPE	AF309385	
Parviphycus pannosus	Spain		AF320983	
Pterocladiella capillacea	Japan		AB023850	
Pterocladia lucida	New Zealand	ASD141	AY648025	
Ptilophora leliaertii	South Africa		AY344047	
Gelidium corneum	Moracco	G2583	HM629821	
Gelidiella fanii	Philippines	G5202		HM026522
Gelidiella fanii	Indonesia	G5136		HM026520
Gelidiella acerosa	Taiwan	G5027		HM120420
Gelidiella acerosa	Taiwan	G5033		HM120420
Gelidiella acerosa	Hawaii, USA	ARS02617		QH423118
Gelidiella acerosa	Hawaii, USA	ARS02612		QH423120
Gelidiella acerosa	Poert Dickson Malaysia	PSM12500	89-rbcL	89-coxI
Gelidiella acerosa	Port Dickson, Malaysia	PSM12500	90-rbcL	90-coxI
Gelidiella acerosa	K. Terrenganu, Malaysia	PSM12670	315-rbcL	315-coxI
Parviphycus sp.	Teluk Kemang, Malaysia	PSM12594	274-rbcL	274-coxI
Parviphycus sp.	Teluk Kemang, Malaysia	PSM12594	274-1-rbcL	
Parviphycus sp.	Port Dickson, Malaysia	PSM12563	222-rbcL	
Parviphycus sp	Port Dickson, Malaysia	PSM12563	222-rbcL	
Parviphycus sp.	K.Terrenganu, Malaysia	PSM12647	319-rbcL	
Parviphycus sp.	K. Terrenganu, Malaysia	PSM12639	310-rbcL	
Parviphycus sp.	K. Terrenganu, Malaysia	PSM12612		285-coxI
Parviphycus sp.		PSM12612		285-coxI